ABSTRACT

Title: DEFINING THE HEMOSTATIC RESPONSE TO AN ORAL FAT LOAD BEFORE AND AFTER EXERCISE TRAINING.

Chad Michael Paton, Doctor of Philosophy, 2005

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INTRODUCTION: Chronic hypertryglyceridemia is thought to be atherogenic and is associated with an elevated thrombotic potential. Aerobic exercise training is known to reduce plasma triglyceride (TG) levels and the purpose of this study was to determine the effect of a single, high-fat meal on markers of inflammation, coagulation, and fibrinolysis before and after exercise training.

MATERIALS and METHODS: Eight subjects were tested for aerobic capacity, body composition, and postprandial lipemia (PPL), followed by 6-months of exercise training and final testing. Blood samples were obtained every 30-minutes following the lipemic challenge for measurement of free fatty acid (FFA), TG, insulin (Ins), and glucose (Glu). Hemostatic variables including factor VII activity (FVIIa), tissue factor pathway inhibitor-factor Xa complex (TFPI/Xa), and plasminogen activator inhibitor-1 (PAI-1) antigen / activity were assessed at 0, 2, and 4 hours postprandial, as well as leukocyte interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and PAI-1 gene expression among 4 subjects during the lipemic challenge.

RESULTS: The exercise training was of sufficient intensity to increase aerobic capacity ($p < 0.0001$) and improve body composition ($p = 0.04$). There were no

differences between tests among PPL responses of FFA, TG, Ins, or Glu, however the main effect mean TG response averaged across all time-points was lower at final testing (139 ± 19 mg•dl^{-1}) versus baseline (154 ± 24 mg•dl^{-1}) ($p = 0.02$). Furthermore, the 4-hour averages for total fat oxidation rate increased by 68% ($p = 0.01$) and total carbohydrate oxidation rate decreased by 29% ($p = 0.009$) from baseline to final testing. IL-6 and PAI-1 gene expression were undetectable in the Paxgene® blood samples, however PAI-1 antigen / activity, FVIIa, TFPI/Xa, and TNF-α gene expression were all improved following exercise training after adjusting for confounders.

CONCLUSION: Aerobic exercise training reduces the potential for coagulation, improves fibrinolytic potential, and reduces leukocyte TNF-α gene expression following the ingestion of a high fat meal.

DEFINING THE HEMOSTATIC RESPONSE TO AN ORAL FAT LOAD BEFORE AND AFTER EXERCISE TRAINING.

By

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Dedication

For my wife, Jamie Cooper

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Chapter 1: Introduction

Atherosclerosis is now considered to be a low-grade inflammatory disease that results in endothelial cell dysfunction. Recent evidence has shown that hypertriglyceridemia, whether chronic or postprandial, is associated with leukocyte activation and an elevated potential for thrombosis, thereby promoting endothelial cell dysfunction and vascular damage (1;2). Although the underlying mechanisms are not completely understood, it has been shown in leukocyte and endothelial cell cultures incubated with triglyceride (TG) rich chylomicrons and very low-density lipoprotein (VLDL), that free fatty acid (FFA) and TG uptake activates factor VII (FVII), tissue factor (TF) (3;4) and plasminogen activator inhibitor-1 (PAI-1) (3;5;6). Furthermore, a VLDL response element in the promoter region of the PAI-1 gene has been identified and shown to increase PAI-1 gene transcription following VLDL binding in vitro (7). Thus, elevated TG and FFA appear to promote atherosclerosis, at least partially, by increasing thrombotic activity and decreasing fibrinolytic activity in leukocytes and endothelial cells.

Although exercise training improves a wide range of cardiovascular disease (CVD) risk factors, the results of studies examining the effects of exercise training on inflammation, coagulation, and fibrinolysis have been inconsistent (8-12). This may be due to the fact that the majority of previous studies measured the various hemostatic markers during resting or fasting conditions. This would not give a complete picture of the capacity of either system because the hemostatic mechanisms are designed to activate in response to a stimulus. Our group and others have found that following a high fat meal, FFA and TG clearance are improved after exercise

training (13;14), and these results lead to the hypothesis that the reduction in FFA and TG levels after exercise training will reduce inflammation and coagulation and increase fibrinolysis following a high-fat meal. We hypothesize that measurements before and after a high fat meal will serve to provide a more valid description of the effect of exercise training on the chronic status of the coagulation and fibrinolytic systems.

Studies examining the acute effects of a high-fat meal on coagulation and fibrinolysis have shown that TF and FVII activation increases (15) with an unexplained paradoxical decrease in plasma PAI-1 levels following the meal (16;17). This may be explained by two factors: first, following the meal, hepatic blood flow increases, which increases the clearance rate for PAI-1; and second, PAI-1 concentrations are highest in the morning, but due to diurnal variations, they decrease throughout the day. Taken at face value, these results suggest that, although a high-fat meal increases the potential for thrombosis, there is an inherent cardio-protective effect because of the increased fibrinolytic potential. The discrepancy between these results and the results of in vitro studies, in which PAI-1 gene expression was increased following TG-laden VLDL binding to leukocytes, illustrates the need to resolve the local (i.e. leukocyte) versus systemic fibrinolytic response (i.e. plasma concentrations) following a high fat meal. Therefore, the purpose of this study was to measure in addition to plasma protein concentrations, PAI-1, interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) gene transcription in leukocytes following the high-fat meal.

Purpose of the study

The purpose of this study was to determine the effect of a single, high-fat meal on the potential for coagulation and leukocyte gene expression before and after exercise training.

Significance of the study

This in vivo model could partially explain the discrepancy between the in vitro and in vivo results of previous studies on systemic fibrinolytic changes following a fat meal. Similarly, it provides a more relevant model through which to study the effects of exercise training on a variety of hemostatic measures. Since vascular thrombosis is responsible for a large portion of CVD and stroke related events, this investigation will lead to a better understanding of the methods by which exercise training reduces CVD and stroke related morbidity and mortality.

Hypotheses

Hypothesis #1: A single high-fat meal will increase plasma factor VII activation (FVIIa) and tissue factor pathway inhibitor-activated factor X (TFPI/Xa) levels, decrease PAI-1:ag and activity levels, and increase leukocyte IL-6, TNF-α, and PAI-1 gene expression.

Hypothesis #2: Post-Prandial FVIIa and TFPI/Xa levels and leukocyte IL-6, TNF-α, and PAI-1 gene expression will increase and PAI-1 activity and antigen will

decrease to a lesser extent after exercise training compared to the sedentary state due to an increase in FFA and/or TG clearance.

Chapter 2: Methods and Procedures

Study Overview: Healthy sedentary middle- to older-aged men and women classified as pre-hypertensive to stage 1 hypertensive with systolic blood pressure of 120-159mmHg, and/or a diastolic blood pressure of 80-99mmHg were screened into the study. Subjects completed a 6-week Dietary Stabilization Period to ensure that all subjects followed the AHA Dietary Guidelines for the General Population. Following the DSP, they underwent baseline testing for aerobic capacity (VO_2max), body composition, and postprandial lipemic measurement of inflammation, coagulation, and fibrinolysis, followed by 6-months of exercise training and final testing.

Unique Aspects of this Trial: Important and unique aspects of this study are (a) a more complete assessment of the change in PAI-1 levels during postprandial lipemia by measuring gene expression, (b) careful control of diet and disease, (c) implementation of a highly standardized exercise training intervention, (d) measurement of inflammation, coagulation, and fibrinolysis following a high-fat meal, and (e) specific, testable, directional hypotheses concerning the effects of exercise training without substantial weight reduction on inflammation, coagulation, and fibrinolysis.

Subjects

Fifty to 75 year old men and women responding to advertisements were contacted by telephone to determine their eligibility and interest. With the exception of age, the subjects sampled represented the general population and included men and women of all races. Pre-hypertensive to stage 1 hypertensive subjects were studied

because they have been shown to be at risk for an impaired hemostatic profile (18-20) and because this project is part of a larger ongoing study assessing the effects of exercise training on hypertension. Subjects were: sedentary (regular aerobic exercise $\leq$2 times/wk and <20 min/session, sedentary occupation); nonsmokers; non-diabetic; and not on lipid-, glucose-, or blood pressure-lowering medications, and not receiving anti-coagulant therapies. Subjects also did not have any other medical conditions that prevented engagement in vigorous exercise. Subjects had a body mass index <37 kg/m^2 so that the physical limitations of excessive obesity would not impede their ability to exercise vigorously. Women were postmenopausal (absence of menses for >2 yrs) with a serum FSH level >30 IU/ml and agreed to maintain their hormone replacement regimen (HRT), either on or not on HRT, constant for the duration of the study. Subjects were screened to exclude those with gastrointestinal disorders, lactose intolerance, or gall bladder dysfunction. Suitable subjects were mailed a medical history questionnaire and scheduled for Screening Visit #1.

Subject Screening

All potential subjects underwent two screening visits prior to entry into the study.

Screening visit #1

The study protocol and informed consent was approved by the University of Maryland College Park Institutional Review Board and each subject reported to the laboratory and their informed consent was obtained. Subjects had their medical history questionnaire reviewed and BMI measured to verify it is <37 kg/m^2. Subjects

were excluded from the study if they had a hematocrit <35, evidence of renal or liver disease, or if they had fasting plasma glucose >126 mg/dL. Lastly, subjects were required to have a fasting plasma glucose value <126 mg•dL-1 and a 2-Hr 75 gram OGTT glucose value of <200 mg•dL-1 to officially enter the study, given that diabetes can affect responses to exercise training.

Screening visit #2

Subjects qualified to this point had a physical and cardiovascular (CV) examination by a physician to detect CV, pulmonary, or other chronic diseases that would preclude exercise testing or training (21). They underwent a Bruce maximal treadmill exercise test (22) to ensure they had no evidence of overt CV disease. Blood pressure, heart rate, and ECG were recorded before the test, at the end of every exercise stage, and every other minute for 6 minutes after exercise. The test was terminated when the subject could no longer continue or CVD signs or symptoms occurred (21). Subjects had <2 mm ST-segment depression and no CVD signs or symptoms (21) during this test to be included in the study.

Dietary Stabilization

Subjects qualified based on the results of the 2 Screening Visits detailed above then entered the dietary stabilization phase of the protocol. Our goal was to examine the effects of exercise training on inflammation, coagulation, and fibrinolysis independent of dietary changes and differential dietary interactions with exercise training. Therefore, we only recruited subjects who agreed to be instructed on and maintain the AHA Dietary Guidelines for the General Population (23) throughout the study in order to minimize the confounding effects of diet between individuals.

Subjects received instruction at 2 sessions/wk for 6 wks by a Registered Dietician in the principles, application, and maintenance of the AHA Dietary Guidelines for the General Population (23-25). They later completed food records after 3 and 6 wks of the dietary stabilization period to assess compliance.

Baseline Testing

After Dietary Stabilization, subjects underwent baseline testing to measure postprandial lipemic challenge measures of inflammation, coagulation, and fibrinolysis. Additionally they were tested for, body composition, VO_2max, and fasting lipoproteins. Further details of these tests are presented below.

Plasma lipoproteins

Following the dietary stabilization period, baseline measurements of plasma total cholesterol, TG, low density lipoprotein (LDL), total high density lipoprotein (HDL), and HDL subfractions (HDL_2 and HDL_3) were analyzed in a Center for Disease Control (CDC)-certified lab using a Hitachi 717 auto-analyzer. The values were averaged from blood samples obtained on two separate days and if the difference between measures was greater than 10%, a third sample was used. LDL was estimated using a previously developed method (26) and total HDL was measured after precipitation with dextran sulfate. HDL_3 was separated using a second high-molecular-weight dextran sulfate precipitation and the HDL_2 fraction was obtained be the difference between total HDL and HDL_3.

Postprandial lipemia test

Subjects were free from infection, fever, cold, or any other illness for at least one week and abstained from taking any medications (including aspirin or other NSAID's), vitamins, herbal supplements, and/or alcohol for two days prior to the postprandial lipemia test (PPLT). For the baseline PPLT, subjects did not engage in physical activity for at least five days prior to testing, as acute exercise has been shown to affect FFA and TG clearance (27-29). For final PPLT testing, subjects performed their last bout of exercise 24-36 hours before the PPLT as the inclusion of acute exercise more accurately represents their current daily condition during exercise training.

The standard liquid fat meal was developed by Patsch et al. (30), and it consists of 386 gm per 2 m^2 of body surface area (BSA), where BSA in $m^2 = 0.00949 \times [(\text{weight in kg})^{0.441}] \times [(\text{height in cm})^{0.655}]$. Of the 386 gm, 325 gm are from heavy whipping cream, 14 gm from granulated sugar, 39 gm from chocolate syrup, and 8 gm from non-fat powdered milk. The total energy content of the 386 gm per 2 m^2 meal is 1362 kilocalories, of which 84% are from fat, 2.8% are from protein, and 14% are from carbohydrate. Subjects reported to our laboratory for testing and consumed the meal within 3 minutes between 0700 and 0900 to minimize the effect of diurnal variation on the markers of inflammation, coagulation, and fibrinolysis.

Expired gases

Expired gases were collected into Douglas bags in 5-min intervals for a total of 20-min prior to meal ingestion, and at 2- and 4-hours after meal ingestion. Each 5-min bag was analyzed for gas content (O_2, CO_2, and N_2) using a medical gas analyzer

(Perkin Elmer, Danbury, CT.) and volume. Respiratory exchange ratio, carbohydrate (CHO), lipid oxidation rate, and total energy expenditure (TEE) were estimated using previously established criteria (31).

Blood sampling

An intravenous catheter was placed in an antecubital vein and blood samples were obtained before ingestion of the fat meal, and every 30-minutes for 4-hours after the meal for the determination of plasma metabolic factors (insulin, glucose, FFA, and TG). Plasma samples for the determination of markers of coagulation and fibrinolysis were collected from the indwelling catheter immediately after placement into the antecubital vein and at 2-, and 4-hours after ingestion of the meal. Whole blood was collected from the indwelling catheter into PAXgene® blood collection tubes (Qiagen Inc., Valencia, CA) immediately following placement of the indwelling catheter before ingestion of the meal and 2-, and 4-hours after ingestion for the purpose of measuring leukocyte PAI-1 and inflammatory gene expression.

Whole blood was collected into 3-ml serum tubes and allowed to coagulate for 30-minutes at room temperature for FFA and 5-ml EDTA tubes for TG measurements. Serum and EDTA whole blood used for plasma metabolic factors was centrifuged at 1,800 x g for 20-minutes, and the serum/plasma was separated and stored in aliquots at -80ºC until assayed. Whole blood for the measurement of FVIIa, TFPI/Xa, and PAI-1:ag and activity concentrations were centrifuged at 3,000 x g for 20-minutes and plasma was separated and re-spun for 5-minutes at 3,000 x g to obtain platelet poor plasma that was stored at -80ºC in separate aliquots until assayed. No

more that one freeze-thaw cycle was allowed for plasma samples of hemostatic variables.

Serum and EDTA plasma were analyzed for changes in FFA (NEFA C, Wako Chemicals, Richmond, VA), TG (Sigma Diagnostics, St. Louis, MO), insulin (Ins) (Linco Research, St. Charles, MO), and glucose (Glu) (YSI 2300 Stat Plus, Yellow Springs, Ohio) concentrations. High sensitivity C-reactive protein (hsCRP) (Alpha Diagnostic International, San Antonio, TX), FVIIa and TFPI/Xa (Imubind, American Diagnostica, Greenwich, CT), and PAI-1:ag (Zymutest, Diapharma, West Chester, Ohio) were measured by enzyme linked immunosorbent assay (ELISA) and PAI-1 activity (Spectrolyze pL, American Diagnostica, Greenwich, CT) was measured by an amydolytic activity assay. All samples were measured in duplicate within the same run in order to minimize variation among subjects (Table 2).

Body composition assessment

Each subject's percent total body fat and lean body mass was measured by dual energy x-ray absorptiometry. Each subject's intra-abdominal visceral and subcutaneous adipose tissue areas were quantified midway between the 4^{th} and 5^{th} lumbar vertebrae using a standardized computed tomography (CT) scan protocol used previously in our laboratories (32). Percent total body fat and visceral fat were used as potential covariates in our analyses to determine the independent effect of exercise training on changes in inflammation, coagulation, and fibrinolysis, as differential changes in total and regional body composition could independently affect thrombotic and fibrinolytic changes with exercise training (33).

Aerobic capacity measurement

All subjects underwent a second maximal treadmill exercise test to assess VO_2max as an index of CV fitness. The test started at 70% of the peak heart rate achieved on the subject's screening exercise test and treadmill grade increased by 2% every 2 minutes (34). Blood pressure, heart rate, and ECG were monitored and the test was terminated when the subject could no longer continue. VO_2 was measured continuously throughout the test and standard criteria were used to determine if a true VO_2max had been achieved (21). VO_2max was measured to derive valid exercise prescriptions for the exercise training intervention and to quantify generalized CV training adaptations.

Whole blood gene expression

Blood samples were obtained from four subjects during the postprandial lipemia test prior to meal ingestion, and at 2- and 4-hours postprandial. However, one subject's 4-hour sample was lost due to centrifugation prior to incubation, thus data for 3 subjects was available for the 4-hour time-point. RNA was extracted according to the manufacturer's recommendation (Paxgene Blood RNA Kit, Qiagen Inc., Valencia, CA) and using the optional on-column DNase treatment (RNase free DNase set, Qiagen Inc., Valencia, CA). Total RNA was collected and prepared in 80μL aliquots, quantified by absorbance at 260 nm (1 absorbance unit = 40μg/ml) and the purity was estimated using the A_{260}/A_{280} ratio in triplicate. 200 ng of total RNA was used for the reverse transcription reaction using random oligonucleotide primers into a final reaction volume of 20μL. Samples were then diluted 1:1 with 20μL of PCR grade H_2O into a final volume of 40μL.

Real time PCR (rt-PCR) was carried out using a Roche Lightcycler (Roche, Mannheim, Germany) with primer/probe sets obtained from TIB (TIB Molbiol, Adelphia, NJ) for PAI-1, IL-6, TNF-α, and RNA polymerase II (RNA pol II) mRNA (Table 1). The master mix for the PCR reactions was obtained from Roche Diagnostics and all reactions were carried out according to the manufacturer's recommendations in triplicate. No template controls (NTC) and no amplification controls (NAC) were included in each run to verify evidence of genomic DNA contamination within master mix (NTC) or samples (NAC). Post PCR products were run on a 3% agarose gel and compared to a standard 50 bp ladder to qualitatively assess product presence and length. Relative expression, using a calibrator (pooled subject sample) normalized to a house keeping gene (RNA pol II) was carried out using the $^{\Delta\Delta}C_T$ method ($2^{-\Delta\Delta C_T}$), [where $^{\Delta\Delta}C_T = {}^{\Delta}C_{T(sample)} - {}^{\Delta}C_{T(calibrator)}$ and $^{\Delta}C_T = C_{T(target\ gene)} - C_{T(HS\ gene)}$] (35) using the manufacturer's software (Roche Lightcycler, Mannheim, Germany).

Exercise Training Intervention

All subjects underwent 3 exercise training sessions/wk supervised by study personnel. The training program lasted 6 months to ensure adequate time for training-induced improvements in the cardiovascular and metabolic systems. Initial training sessions consisted of 20 min of exercise at 50% VO_2max and increased by 5 min every wk until 40 min of exercise at 50% VO_2max are completed each session. Training intensity then increased by 5% VO_2max every wk until an intensity of 70% VO_2max is achieved. Subjects added a lower intensity unsupervised 45-60 min walk

on the weekend after 10 wks of training and recorded in printed logs their exercise heart rate, duration, and mode information for all supervised and unsupervised training sessions. Adherence to the training prescription was assessed for every exercise training session by inspecting training log exercise intensity, duration, and frequency data.

Dietary monitoring

Our goal was to examine the effects of exercise training on the markers of inflammation, coagulation, and fibrinolysis following an oral fat load independent of changes in diet composition and diet-induced weight loss. We did not recruit subjects seeking to lose substantial amounts of weight and therefore, subjects were instructed to maintain their habitual caloric intake during the study while following the AHA Dietary Guidelines for the General Population (23). Subjects were weighed weekly and those losing more weight than expected from their exercise energy expenditure were counseled to increase their caloric intake back to initial levels. Subjects also completed 7 day food records every 2 months during the intervention to assess compliance with the AHA Dietary Guidelines for the General Population. If dietary changes occurred, subjects were counseled by the RD on how to resume the AHA Dietary Guidelines. In addition to counseling and diet records, subjects were provided low-fat, low-sodium snacks after each exercise training intervention to replace the calories expended during physical activity. Body weight was measured each week and monitored by study personnel to ensure that subjects remained within the required weight range. If subjects gained or lost more than 5% of their initial body weight, they were dropped from the study.

Final Testing

At the completion of the exercise training intervention, subjects completed 7 day food records to insure dietary compliance prior to reassessment of postprandial lipemic measures of inflammation, coagulation, fibrinolysis, body composition, VO_2max, and fasting plasma lipoproteins. Subjects continued their exercise training until all final testing was completed. Each subject underwent the final postprandial lipemia test 24-36 hours after their last exercise session.

Statistics

A two-factor (test x time) repeated measures ANOVA using a heterogeneous compound symmetry matrix was used to analyze the interaction effects for changes in plasma coagulation and fibrinolytic variables, as well as leukocyte TNF-α gene expression. Statistical analyses were performed using SAS software (SAS version 8.2, SAS institute Inc., Cary, NC). The *a priori* alpha level was set at $p<0.05$ for all planned comparisons. Analysis of the residual variance was conducted to ensure a normal distribution was present. Fasting plasma levels of cholesterol fractions, body composition, and lipemic measures were included as potential covariates (where physiologically appropriate, see below) in the analyses. All data were normally distributed, with the exception of PAI-1:ag (non-normal residual variance distribution) which was $\log_{10}$ transformed prior to statistical analyses. All data are represented as mean ± SE including those for PAI-1:ag, although the probability values for the latter are from the $\log_{10}$ transformed data.

Potential covariates for the selected variables measured in this study include body composition measures (total fat and intra-abdominal fat), fasting lipoproteins (LDL, HDL_3), and postprandial lipemic measures (hsCRP, FFA, Ins, and lipid oxidation rate). A maximum of four potential covariates were included for each variable, thus only the most biologically reasonable variables were used. Non-significant ($\alpha = 0.05$) covariates were removed from the model one at a time, starting with the least significant and ending when all remaining were significant.

Chapter 3: Results

Exercise training intervention

A total of 8 subjects completed all aspects of the exercise training intervention and PPLT testing. Although the intervention was of sufficient frequency, intensity, and duration to elicit improvements in VO_2 max, body composition, and total cholesterol, there were no improvements in fasting hsCRP, TG, LDL, HDL, or HDL sub-fractions (Table 3) with six months of training. All subjects maintained initial total body weight throughout the intervention, despite a slight, but not significant decrease in the mean body mass at final testing (-1.3 kg).

Postprandial lipemia test

There were no differences between fasting measures of FFA, TG, Ins, or Glu at baseline versus final testing. There was a significant increase in plasma FFA, TG, and Ins levels during the PPLT at 2- and 4-hours after meal consumption before and after exercise training (Table 4). There were no significant differences in the postprandial levels at each time point between the two tests, although the time

averaged mean postprandial TG levels were lower at final testing (139 ± 19 mg•dl^{-1}) versus baseline (154 ± 24 mg•dl^{-1}) ($p = 0.02$). There was no significant difference between time averaged FFA ($p = 0.27$) or Ins ($p = 0.17$) levels between the two tests. Plasma glucose levels remained unchanged throughout each test; however the average CHO and lipid oxidation rates over the 4-hr period were altered following exercise training (Figure 1). The average CHO oxidation rate across the entire 4-hr postprandial lipemia test significantly decreased from 282 ± 48 to 201 ± 26 mg•min^{-1} ($p = 0.009$) and total lipid oxidation during the same period increased from 10.5 ± 13.1 to 32.8 ± 9.3 mg•min^{-1} ($p = 0.01$), while average total energy expenditure remained unchanged (1.14 ± 0.05 vs. 1.07 ± 0.09 kcal•min^{-1} $p = 0.29$) between the two tests.

Plasma coagulation and fibrinolytic measures

Before exercise training, $\log_{10}$ PAI-1:ag levels significantly decreased from fasting to 2-hrs postprandial ($p = 0.001$) with a non-significant trend toward a further reduction from 2- to 4-hrs ($p = 0.07$), and at 4-hrs post-prandial, $\log_{10}$ PAI-1:ag levels were significantly lower than fasting ($p < 0.0001$) (Table 5). After exercise training, $\log_{10}$ PAI-1:ag levels did not significantly change from fasting to 2-hr ($p = 0.10$) but decreased from 2- to 4-hr ($p = 0.0007$) and 4-hrs was lower than fasting ($p < 0.0001$). At final testing, $\log_{10}$ PAI-1:ag levels were lower in the fasted state, not significantly different at 2-hrs, and significantly lower at 4-hrs postprandial versus baseline (Figure 2).

There was no change in PAI-1 activity from fasting to 2-hours postprandial before or after exercise training after adjusting for intra-abdominal fat and lipid

oxidation rate, however during both tests there was a significant decrease from 2- to 4-hours ($p = 0.003$ baseline; $p < 0.0001$ final). There was no difference in the fasting measures of PAI-1 activity between tests, however after exercise training the 2-hr and 4-hr postprandial PAI-1 activity levels were significantly lower than baseline (2-hr $p = 0.03$; 4-hr $p = 0.001$) (Figure 3).

After adjusting for LDL and lipid oxidation rate, before exercise training FVIIa levels increased from fasting to 2-hrs postprandial ($p < 0.02$) and decreased significantly from 2- to 4-hrs ($p = 0.03$) where it was no longer elevated compared to fasting levels ($p = 0.39$). At final testing, FVIIa levels significantly decreased from fasting to 2-hrs ($p = 0.008$), increased significantly from 2- to 4-hrs ($p = 0.004$), and was not different from fasting at 4-hrs postprandial ($p = 0.06$). FVIIa was not different between tests prior to meal ingestion, however at 2- and 4-hrs, FVIIa was significantly lower at final testing compared to baseline (Figure 4).

TFPI/Xa levels at baseline testing did not change during the 4-hr postprandial lipemia test after adjusting for LDL and FVIIa levels (0- vs 2-hr $p = 0.37$; 0- vs 4-hr $p = 0.29$; 2- vs 4-hr $p = 0.07$). However, at final testing, there was a significant increase in TFPI/Xa levels from fasting to 2-hrs ($p = 0.001$) and from fasting to 4-hrs ($p = 0.01$), with no change from 2- to 4-hrs ($p = 0.24$). There was no difference between tests in fasting levels of TFPI/Xa, however at 2- and 4-hrs, TFPI/Xa levels were significantly higher at final versus baseline testing (Figure 5).

Whole blood gene expression

Neither fluorescence nor post-PCR product was detectable for IL-6 or PAI-1 mRNA in the subject samples however, TNF-α and RNA pol II gene expression were

robust and detectable in all subject samples and the data for all four subjects are presented in Figure 6. After adjusting for hsCRP and total body fat mass, TNF-α gene expression increased from fasting to 2-hours postprandial ($p = 0.04$) and significantly decreased from 2- to 4-hours ($p = 0.007$) such that the 4-hr postprandial value was significantly lower than fasting before exercise training ($p = 0.02$). At final testing, TNF-α gene expression increased from fasting to 2-hours ($p = 0.02$) and remained significantly elevated at 4-hours versus the fasting level ($p = 0.04$). Between tests, fasting TNF-α gene expression was lower at final testing ($p = 0.0007$), lower at 2-hours postprandial ($p = 0.006$), and not different at 4-hours ($p = 0.17$) versus baseline.

Chapter 4: Discussion

Exercise training intervention

The present study was designed to assess the changes in the hemostatic response to a postprandial lipemia test before and after 6-months of aerobic exercise training. We also sought to minimize the amount of weight lost during the study intervention, as weight loss has been shown to affect hemostasis and inflammation independent of metabolic and cardiovascular adaptations (36;37). Despite the fact that there were no improvements in lipoprotein subfractions or fasting insulin levels, the exercise training intervention was of sufficient intensity to elicit significant improvements in aerobic capacity and body composition.

Postprandial lipemia test

The standard fat meal employed in the current study design sufficiently induced a lipemic response; however there were no differences between tests (baseline vs. final) among postprandial values for FFA, TG, Ins, or Glu at any single time-point (0-, 2-, or 4 hours). There was a significant reduction in the average TG response across all time-points from baseline to final testing which is in agreement with previous studies (27;28). Others have reported significant improvements in the postprandial lipemic response following acute bouts of physical activity, with the majority of the improvements observed within 12-24 hours after exercise. Our study assessed the response at 24-36 hours following the last bout of exercise which may explain the fact that no differences were observed in these variables and confirms a

previous 12-week exercise training study in which there was no reported reduction in postprandial lipemia 48-hours after a single bout of acute exercise (38).

The major factor influencing the improved lipemic response following exercise training is believed to be an increase in lipoprotein lipase (LPL) activity that has been shown to be transient, with the largest increase in activity occurring around 18-hours after exercise (39;40). Another and less transient factor that is associated with aerobic exercise training is an improved skeletal muscle β-oxidative capacity and we observed a significant decrease in the average rate of CHO oxidation with a concomitant increase in lipid oxidation during the postprandial lipemia test following exercise training.

Unlike the transient nature of skeletal muscle LPL activity, the increase in β-oxidative capacity remains elevated for days or weeks with chronic aerobic exercise training. We hypothesized that the exercise training-mediated improvements in inflammation and hemostasis would be due to improvements in FFA and TG clearance; however the results of this study imply that the improvements may be due to increased FFA oxidation. It is known that the tissue type involved in the uptake and clearance of FFA, TG, and Glu from plasma may contribute to the inflammatory and hemostatic response to an oral fat load (41). In the sedentary state, more of the ingested TG is directed to adipocyte storage which has been shown to increase the expression of inflammatory cytokines and PAI-1 levels (42). Thus, with a larger amount of the ingested fat being directed toward β-oxidation in the trained state, adipocyte-directed storage cytokine/PAI-1 expression would be decreased.

Finally, it is not unreasonable to assume that with such a large amount of ingested fat in a single meal, enterocyte chylomicron synthesis and secretion may be occurring at near maximal capacity. In this case, the postprandial levels of plasma FFA, TG, and Ins would not change over a 4-hour time period before versus after exercise training due to the continued enterocyte FFA uptake, chylomicron secretion, and plasma appearance of TG. Thus, the ability to determine changes in plasma FFA and/or TG clearance may be delayed until complete intestinal clearance of the ingested meal has occurred. Many previous studies have assessed the postprandial lipemic response for 8+ hours and have shown that the peak lipemic response tends to be at 4-hours. It is therefore possible that differences between time-points may exist beyond the peak response although the usefulness of measurements beyond 4-hours is in question as people seldom go more than 4-hours between meals.

Plasma coagulation and fibrinolytic measures

One of the purposes of this investigation was to more accurately define the effect of aerobic exercise training on hemostasis by measuring the response of the system under stress. The majority of previous studies that have attempted to show a reduction in coagulation potential following long-term engagement in physical activity or exercise training have done so using an assessment of coagulation factors in the fasted state and have consistently failed to show a clear reduction (8-12;43). This is likely due to the fact that the hemostatic response functions over a wide range and many of the individual factors are capable of increasing their activity to more than 100% over resting levels (44). It would be unreasonable to assume that a clear reduction could be apparent when assessing the system within the lowest range of its

activity. This is confirmed by the results of the present study in which the levels of FVIIa and TFPI/Xa showed no differences in the fasted state between tests (baseline vs. final), yet there was a clear difference between tests after the fat meal.

There is very little known about the effect of exercise training on anti-coagulant activity, with only one study published in which TFPI levels were measured before and after exercise training. The authors reported no change in fasting levels of circulating TFPI among diabetic subjects (45), although circulating TFPI is not believed to be a good predictor of functional or total TFPI, which is why we chose to measure the TFPI/Xa complex. The majority of functional TFPI is believed to be bound to the endothelium where it is capable of binding to the TF-FVIIa-FXa ternary complex and rapidly terminating the activation of coagulation. Very little is known about the effect of exercise training on plasma TFPI levels, and nothing is known about changes in endothelial cell expression of TFPI with training. Here we show an increase in TFPI-mediated inactivation of the extrinsic pathway after training, and although it is beyond the scope of this investigation, it is tempting to speculate that a higher quantity of TFPI may have been expressed on the endothelial cell surface after training.

As with coagulation potential, previous studies on the effect of exercise training on fibrinolytic potential have either reported decreases or no change in PAI-1 antigen or activity levels (46;47). The two most logical reasons for the disagreement between these previous results are: 1) variation in exercise training frequency, intensity, and/or duration, and 2) measurement of fasting PAI-1 levels. In the present study, we employed a long-term relatively high intensity exercise training program

and we measured the response of PAI-1 following a meal challenge. As a result, we were able to illustrate a clear reduction in postprandial levels of PAI-1:ag and activity despite the fact that there was no difference in fasting PAI-1 activity levels between tests.

Another main outcome of this project was to address the previously noted paradoxical decrease in PAI-1 levels during the postprandial lipemia test. No study to date has attempted to address this discrepancy, which taken at face value suggests that fibrinolytic activity is increased following a high fat meal. The most logical explanation for the resulting decrease in PAI-1:ag is that the rate of hepatic blood flow is higher after ingestion of the meal. In addition to this, PAI-1 activity levels may be decreasing, at least in part, due to diurnal variations with the highest levels of activity in the morning. In light of these facts, it is possible that endothelial, hepatic, or adipocyte PAI-1 release may in fact increase while plasma levels are decreasing. Despite the fact that this paradoxical PAI-1 decrease exists following a fat meal, the fact that postprandial PAI-1:ag and activity were lower after exercise training illustrates a clear benefit of aerobic exercise training on reducing the risk for CVD-related outcomes.

Whole blood gene expression

We sought to examine the effect of exercise training on the postprandial response of leukocyte gene expression following a high fat meal. Our aim was to determine if PAI-1 and inflammatory gene expression were increased despite the paradoxical decrease in circulating PAI-1 protein levels. Unfortunately, the blood collection tubes that were used for the stabilization of mRNA prevented the

measurement of both IL-6 and PAI-1 gene expression and we were unable to address this aspect of the investigation. This was because monocytes comprise a small fraction of the whole blood leukocyte population (0-9%) and without separation of cell types prior to RNA isolation, they provide an equally low contribution of mRNA to the total mRNA obtained. It was understood at the onset of the investigation that monocytes would be the only cells expressing IL-6 and PAI-1 (48), however we believed that their mRNA would still be detectable even in the presence of whole blood leukocyte mRNA. Based on the results of this study, and others (49-52), it is apparent that an investigation of monocyte-derived mRNA should be conducted only after separation of mononuclear cells and whole blood gene expression should be avoided.

In addition to monocytes, TNF-α is expressed in multiple mononuclear blood cell types including B cells, T cells (53), and neutrophils (53;54). Consequently, its expression was robust in the RNA samples obtained and we were able to show an improvement in inflammation at rest and following a meal challenge due to aerobic exercise training. While the high fat meal increased leukocyte TNF-α gene expression from fasting to 2-hours postprandial during both tests, the degree to which gene expression increased in the trained state was lower than baseline. We are unable to determine whether the observed changes in TNF-α gene expression resulted in comparable changes in plasma TNF-α levels, nevertheless it is reasonable to conclude that the leukocyte TNF-α response to the high fat meal was lower after training versus baseline.

Summary

Given that the majority of life is spent in the postprandial state and because the inflammatory and hemostatic systems function over a wide range of values in response to stimuli, assessment of their function in the fasted state does not provide an accurate description of the effect of exercise training. However, the results of this investigation provide clear evidence that inflammation and the coagulation and fibrinolytic potentials are improved with aerobic exercise training. In addition, by reducing or eliminating the increase in coagulation potential and inflammation and increasing fibrinolysis, aerobic exercise training may reduce the risk for CVD- and stroke-related morbidity and mortality. Finally, this is the first investigation into the effect of exercise training on anti-coagulant potential among healthy subjects and the mechanisms responsible for the paradoxical decrease in plasma PAI-1 levels remain to be determined. Future studies into the area of exercise and hemostasis should focus on assessment of the systems under a controlled stress while determining the change in TFPI, antithrombin III, and protein C activities in addition to procoagulant changes.

Appendix A: Limitations

The following limitations apply to the present study:

1. With the exception of evidence of CVD and diabetes, all other personal and medical history was self-reported.
2. Subjects were not screened for other chronic inflammatory diseases, such as hepatitis, that are known to influence hemostasis and leukocyte gene expression.
3. Dietary compliance, although assessed by diet records, was self-reported.
4. No control group was used in the present study and it is possible that seasonal variation could have contributed to the change in hemostatic and inflammatory profiles of the subjects.
5. The blood collection tubes used for the determination of whole blood gene expression do not allow for the separation of mononuclear cell types from blood and therefore, we are not able to determine the cell type(s) from which mRNA was obtained.
6. Circulating leukocytes are obtained from whole blood and it is possible that endothelial cell-bound leukocytes may exhibit a different phenotype than that which was obtained from the circulation.

Appendix B: Delimitations

This project has been delimited in the following areas:

1. This project is designed to provide a more valid assessment of the change in coagulation potential following aerobic exercise training.
2. There is a careful control of diet and disease to eliminate known confounding factors.
3. The project uses the implementation of a highly standardized exercise training intervention to eliminate the potential of differential responses to varying degrees of exercise dosage.
4. Through the measurement of inflammation, coagulation, and fibrinolysis following a high-fat meal, a more valid description of the change in hemostasis and inflammation following exercise training is possible by assessing the system under stress.
5. The effects of exercise training without substantial weight reduction was used to determine the independent effect of aerobic exercise training on inflammation, coagulation, and fibrinolysis.

Appendix C: Human Subject Approval Forms

UNIVERSITY OF MARYLAND
INSTITUTIONAL REVIEW BOARD

June 10, 2004

MEMORANDUM

Approval of Human Subjects Application

TO: Dr. James Hagberg
Mr. Chad Paton
Department of Kinesiology

FROM: Drs. Phylis Moser-Veillon and Marc Rogers
Co-Chairpersons, Institutional Review Board

IRB NUMBER AND PROJECT TITLE:
00741; "ACE Genotype, Blood Pressure, and Exercise Training in Hypertensives"

Enclosed are two copies of the Institutional Review Board (IRB) Approval Document, a copy of the approved consent form, and any copies of your application which are not needed by this office. Please sign one copy of the approval document and return it to this office. **Please note that approval to use this consent form expires on May 31, 2005. If you wish to collect data from human subjects in connection with this research project after this date, please submit a renewal application to the IRB at least 30 days before approval is due to expire.**

We ask that you not make any changes to the approved protocol before this date, without first notifying and obtaining the approval of the Institutional Review Board. Also, please note the following regarding IRB approvals: (1) University regulations require that you use a copy of the attached consent form, containing the approval stamp of the IRB, when conducting your data collection; (2) Any protocol deviations which may occur should be reported to the Institutional Review Board. Thank you.

SPECIAL NOTE REGARDING STUDENT RESEARCHERS:
Unless otherwise requested, the IRB will send approval paperwork to the Principal Investigator. We ask that any student researchers working on this project receive a copy of that paperwork, which they may need in order to apply for graduation. PLEASE BE ADVISED THAT THE IRB MAY NOT BE ABLE TO PROVIDE COPIES OF THE ENCLOSED PAPERWORK, particularly if several years have passed since the date of the original approval.

Institutional Review Board

University of Maryland

College Park, MD 20742

Renewal Application Approval Document for Expedited Review of Non-Exempt Projects

PLEASE NOTE: Institutional Review Board approval of this project expires on June 16, 2005

PRINCIPAL INVESTIGATOR:	Dr. James Hagberg
CO-INVESTIGATOR:	Not applicable
STUDENT INVESTIGATOR:	Chad Paton
ADMINISTERING DEPARTMENT:	Department of Kinesiology
IRB NUMBER & PROJECT TITLE	00741; "ACE Genotype, Blood Pressure, and Exercise Training in Hypertensives"

The University IRB reviewed the above-mentioned project on Monday, June 7, 2004, in accordance with Public Health Service grant policy as defined in "The Institutional Guide to DHHS Policy on Protection of Human Subjects," 12-1-71, and in Title 45, Code of Federal Regulations, Part 46.

University of Maryland, College Park Institutional Review Board

Marc Rogers, Associate Professor, Kinesiology, CO-CHAIRPERSON

Phylis Moser-Veillon, Professor, Nutrition and Food Science, CO-CHAIRPERSON

Kimberly Hale-Cerney, Non-University Member, IRB SECRETARY

Denise A. Andrews, University Counsel, Office of Legal Affairs

Ethelyn Bishop, Non-University Member

Robert Brown, Emeritus Faculty Member, Department of Psychology

Samuel A. Bozeman, M.D., Physician, Health Services, Health Center

Bradley Bunkelow, Associate Professor, Public and Community Health

Jude A. Cassidy, Professor, Psychology

Catherine Doughty, Center for the Advanced Study of Language

Margaretha S. Lucas, Ph.D., Counseling Center

Kenneth Jennings, Jr., Non-University Member

Joan Pontow, M.D., Non-University IRB Member

Eric Wish, Associate Professor, Criminology and Criminal Justice

(Director, Center for Substance Abuse Research)

The IRB effected an independent determination of: (1) the rights and welfare of the individual or individuals involved, (2) the appropriateness of the methods used to secure informed consent, and (3) the risks and potential benefits of the investigation. The IRB has concluded that proper safeguards have been taken by the principal investigator, as stated in the research proposal. The IRB approves this project as conforming to University and Public Health Service Policy in protecting the rights of the subjects.

Marc Rogers

Phylis Moser-Veillon, IRB Co-Chairperson OR **Marc Rogers, IRB Co-Chairperson**

The Principal Investigator (and Co-Investigator and Student Investigator, where appropriate), in signing this report, agree to follow the recommendations of the IRB, to notify the Office of the Vice President of Research of any additions to or changes in procedure subsequent to this review, to provide information on the progress of the research on an annual basis, and to report any instances of injuries to subjects and unanticipated problems involving risks to subjects or others. Any consent forms used in connection with this project must be retained by the Principal Investigator for three years after completion of the research.

_____ and _____

Principal Investigator (or Faculty Advisor) Co-Investigator

_____

Student Investigator

PLEASE RETURN ONE SIGNED COPY TO: *IRB OFFICE, ROOM 2100, BLAIR LEE BUILDING, CAMPUS--- 5121.* Thank you

Institutional Review Board
University of Maryland
College Park, MD 20742

Renewal Application Approval Document for Expedited Review of Non-Exempt Projects

****PLEASE NOTE: Institutional Review Board approval of this project expires on June 30, 2005****

PRINCIPAL INVESTIGATOR:	Dr. James Hagberg
CO-INVESTIGATOR:	Not applicable
STUDENT INVESTIGATOR:	Chad Paton
ADMINISTERING DEPARTMENT:	Department of Kinesiology
IRB NUMBER & PROJECT TITLE	00741; "ACE Genotype, Blood Pressure, and Exercise Training in Hypertensives"

The University IRB reviewed the above-mentioned project on Monday, June 7, 2004, in accordance with Public Health Service grant policy as defined in "The Institutional Guide to DHHS Policy on Protection of Human Subjects," 12-1-71, and in Title 45, Code of Federal Regulations, Part 46.

University of Maryland, College Park Institutional Review Board

Marc Rogers, Associate Professor, Kinesiology, CO-CHAIRPERSON
Phyllis Moser-Veillon, Professor, Nutrition and Food Science, CO-CHAIRPERSON
Kimberly Hale-Carney, Non-University Member, IRB SECRETARY
Denise A. Andrews, University Counsel, Office of Legal Affairs
Ethelyn Bishop, Non-University Member
Robert Brown, Emeritus Faculty Member, Department of Psychology
Sacared A. Bodison, M.D., Physician, Health Services, Health Center
Bradley Boekeloo, Associate Professor, Public and Community Health
Jude A. Cassidy, Professor, Psychology
Catherine Doughty, Center for the Advanced Study of Language
Margaretha S. Lucas, Ph.D., Counseling Center
Kenneth Jennings, Jr., Non-University Member
Joan Pozzow, M.D., Non-University IRB Member
Eric Wish, Associate Professor, Criminology and Criminal Justice
(Director, Center for Substance Abuse Research)

The IRB effected an independent determination of: (1) the rights and welfare of the individual or individuals involved, (2) the appropriateness of the methods used to secure informed consent, and (3) the risks and potential benefits of the investigation. The IRB has concluded that proper safeguards have been taken by the principal investigator, as stated in the research proposal. The IRB approves this project as conforming to University and Public Health Service Policy in protecting the rights of the subjects.

Marc Rogers

Phyllis Moser-Veillon, IRB Co-Chairperson OR **Marc Rogers, IRB Co-Chairperson**

The Principal Investigator (and Co-Investigator and Student Investigator, where appropriate), in signing this report, agree to follow the recommendations of the IRB, to notify the Office of the Vice President of Research of any additions to or changes in procedure subsequent to this review, to provide information on the progress of the research on an annual basis, and to report any instances of injuries to subjects and unanticipated problems involving risks to subjects or others. Any consent forms used in connection with this project must be retained by the Principal Investigator for three years after completion of the research.

_____ and _____

Principal Investigator (or Faculty Advisor) Co-Investigator

_____

Student Investigator

PLEASE RETURN ONE SIGNED COPY TO: *IRB OFFICE, ROOM 2100, BLAIR LEE BUILDING, CAMPUS--- 5121.* Thank you

Appendix D: Definition of Terms*

Atherosclerosis: The progressive narrowing and hardening of the arteries over time.

β-oxidation: The oxidative breakdown of fatty acids into acetyl-coenzyme A by repeated oxidation at the beta-carbon atom.

Emulsification: The process of preparing one liquid distributed in small globules throughout the body of a second liquid.

Esterification: The process of converting an acid into an alkyl or aryl derivative. Most frequently the process consists of the reaction of an acid with an alcohol in the presence of a trace of mineral acid as catalyst or the reaction of an acyl chloride with an alcohol. Esterification can also be accomplished by enzymatic processes.

Fibrinolysis: Solubilisation of fibrin in blood clots, chiefly by the proteolytic action of plasmin.

Gene expression: The full use of the information in a gene via transcription and translation leading to production of a protein and hence the appearance of the phenotype determined by that gene. Gene expression is assumed to be controlled at various points in the sequence leading to protein synthesis and this control is thought to be the major determinant of cellular differentiation in eukaryotes.

Hemostasis: The arrest of bleeding, either by the physiological properties of vasoconstriction and coagulation or by surgical means.

Hypertriglyceridemia: Condition of elevated triglyceride concentration in the blood; an inherited form occurs in familial hyperlipoproteinemia IIb and

hyperlipoproteinemia type IV. It has been linked to higher risk of heart disease and arteriosclerosis.

Inflammation: A localized protective response elicited by injury or destruction of tissues, which serves to destroy, dilute or wall off (sequester) both the injurious agent and the injured tissue. It is characterized in the acute form by the classical signs of pain (dolor), heat (calor), redness (rubor), swelling (tumour) and loss of function (functio laesa). Histologically, it involves a complex series of events, including dilatation of arterioles, capillaries and venules, with increased permeability and blood flow, exudation of fluids, including plasma proteins and leukocyte migration into the focus.

Knock out: Informal term for the generation of a mutant organism in which the function of a particular gene has been completely eliminated (a null allele).

Lipolysis: The breakdown of fat.

Oral fat load: Ingestion of a fatty meal by means of the alimentary canal (mouth).

Postprandial: Occurring after dinner or after a meal (postcibal).

Thrombosis: The formation, development or presence of a thrombus.

Thrombus: An aggregation of blood factors, primarily platelets and fibrin with entrapment of cellular elements, frequently causing vascular obstruction at the point of its formation. Some authorities thus differentiate thrombus formation from simple coagulation or clot formation.

*From Dorland's *Illustrated* Medical Dictionary, 28th Ed. W.B. Saunders Company, Philadelphia, PA, ©1994 , and medical-dictionary.com.

Appendix E: Tables

Table 1. Primers and probes used in quantitative RT-PCR.

Gene (EGI)	Primers (5'-3')	Probes (5'-3')
PAI-1 (5054)	F-ATGGGATTCAAGATTGATGACA R-CAAGTTGCTGATCATACCTTTTG	FL-TGGTGCTGATCTCATCCTTGTTCC LC-GGCCCCATGAGCTCCTTGTACA
IL-6 (3569)	F-CTTTTGGAGTTTGAGGTATACCTAG R-CGCAGAATGAGATGAGTTGTC	FL-AGATGCAATAACCACCCCTGACCCAA LC-CACAAATGCCAGCCTGCTGACGAA
TNF-α (7124)	F-GGCAGTCAGATCATCTTCTCGAA R-CCTTGGTCTGGTAGGAGACG	FL-GCCCCTCCACCCATGTGCTCC LC- CACCCACACCATCAGCCGCATC
RNA pol II (5430)	F-GGCATGTTCTTTGGTTCAGCA R- GGTCATTCCACTCCCAACACT	FL- CTGGTTCCAAGGTGTCATGGCA LC- GAGAGATTCCACCCATGGGACTG

RT-PCR: Real time polymerase chain reaction; EGI: Entrez gene identification number; PAI-1: Plasminogen activator inhibitor-1; IL-6: Iterleukin-6; TNF-α: Tumor necrosis factor-α; RNA pol II: RNA polymerase II. F: Forward (sense) primer; R- Reverse (antisense) primer; FL: Fluorescein labeled FRET hybridization probe; LC: Red 640 labeled FRET hybridization probe.

Table 2: Intra-assay coefficients of variation among measures.

Variable	Intra-assay CV
PAI-1 ag	4.6%
PAI-1 act	5.7%
FVIIa	6.2%
TFPI/Xa	6.9%
TNF-α	0.5%
RNA pol II	0.4%

PAI-1ag: Plasminogen activator inhibitor-1 antigen; PAI-1 act: Plasminogen activator inhibitor-1 activity; FVIIa: Activated factor VII; TFPI/Xa: Tissue factor pathway inhibitor- activated factor X complex; TNF-α: Tumor necrosis factor alpha; RNA pol II: RNA polymerase II.

Table 3. Subject characteristics before and after exercise training.

	Baseline	**Final**	*P (btw tests)*
Age (years)	58.9 ± 4.7	---	---
VO_2 max ($L \cdot min^{-1}$)	2.2 ± 0.2	2.6 ± 0.2	0.007
Total fat (Kg)	30.0 ± 3.6	28.8 ± 3.7	0.04
TW (Kg)	80.4 ± 3.6	79.1 ± 3.6	0.80
hsCRP ($mg \cdot L^{-1}$)	0.87 ± 0.6	0.96 ± 0.6	0.51
TC ($mg \cdot dl^{-1}$)	194 ± 13	169 ± 10	0.004
LDL ($mg \cdot dl^{-1}$)	109 ± 13	99 ± 12	0.56
TG ($mg \cdot dl^{-1}$)	129 ± 23	104 ± 25	0.49
HDL ($mg \cdot dl^{-1}$)	49 ± 6	45 ± 5	0.64
HDL_2 ($mg \cdot dl^{-1}$)	8.9 ± 3.1	8.1 ± 2.8	0.85
HDL_3 ($mg \cdot dl^{-1}$)	40.2 ± 4.1	37.3 ± 2.5	0.53

Data are means ± SE (n=8); Baseline: Before aerobic exercise training; Final: After 6-months of aerobic exercise training; VO_2 max: Maximal oxygen consumption; Total fat: Total body fat mass; TW: Total body weight; hsCRP: High sensitivity C-reactive protein; TC: Total plasma cholesterol; LDL: Low density lipoprotein; TG: Triglyceride; HDL: high density lipoprotein; HDL_2 and HDL_3: HDL sub-fractions.

Table 4. Fasting and postprandial values of TG, FFA, Insulin, and glucose before and after exercise training.

Test	**Time**			Mean differences
	Fasting	2-Hr	4-Hr	within test
TG ($mg \cdot dl^{-1}$)				
Baseline	102 ± 20	155 ± 27	222 ± 33	*0-Hr < 2-Hr< 4-Hr*
Final	93 ± 17	135 ± 18	196 ± 28	*0-Hr < 2-Hr< 4-Hr*
P	*0.48*	*0.25*	*0.19*	
FFA ($mmol \cdot L^{-1}$)				
Baseline	0.36 ± 0.05	0.30 ± 0.04	0.53 ± 0.05	*0-& 2-Hr < 4-Hr*
Final	0.40 ± 0.07	0.31 ± 0.05	0.54 ± 0.04	*0-& 2-Hr < 4-Hr*
P	*0.55*	*0.90*	*0.82*	
Insulin ($pmol \cdot L^{-1}$)				
Baseline	67 ± 10	180 ± 38	136 ± 26	*0-& 4-Hr < 2-Hr*
Final	69 ± 12	162 ± 39	118 ± 18	*0-& 4-Hr < 2-Hr*
P	*0.86*	*0.63*	*0.42*	
Glucose ($mg \cdot dl^{-1}$)				
Baseline	98 ± 3	106 ± 6	98 ± 4	*No sig. dif.*
Final	98 ± 3	105 ± 5	105 ± 5	*No sig. dif.*
P	*0.90*	*0.88*	*0.15*	
CHO ox ($mg \cdot min^{-1}$)				
Baseline	298 ± 66	301 ± 55	248 ± 40	*No sig. dif.*
Final	198 ± 34	203 ± 21	203 ± 31	*No sig. dif.*
P	*0.047*	*0.03*	*0.13*	
Lipid ox ($mg \cdot min^{-1}$)				
Baseline	2 ± 20	-2 ± 17	31 ± 9	*2-Hr < 4-Hr*
Final	26 ± 8	33 ± 12	39 ± 13	*No sig. dif.*
P	*0.16*	*0.02*	*0.50*	

Data are means ± SE (n=8); Test conditions: Baseline (before aerobic exercise training), Final (after 6-months of aerobic exercise training); TG: Triglyceride; FFA: Free fatty acid; CHO ox: Carbohydrate oxidation rate. P represents mean comparisons between tests at each level of time (i.e. fasting baseline vs. fasting final). Mean differences within test represents a significant difference between time points within a single test (i.e. fasting baseline vs. 2-hr baseline vs. 4-hr baseline).

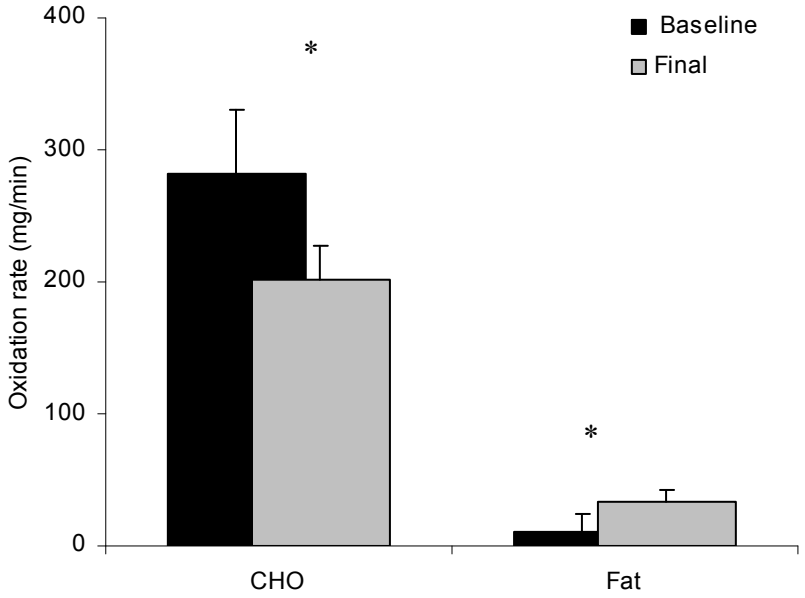
Table 5. Fasting and postprandial values of hemostatic variables before and after exercise training.

Test	**Time**			Mean differences within test
	Fasting	2-Hr	4-Hr	
PAI-1 ag ($ng \cdot ml^{-1}$)				
Baseline	19.1 ± 5.1	12.0 ± 3.1	8.8 ± 2.4	*0-Hr > 2- & 4-Hr*
Final	12.9 ± 2.8	9.8 ± 2.3	5.1 ± 1.4	*0-Hr > 4-Hr*
P	*0.006*	*0.28*	*0.003*	
PAI-1 act ($AU \cdot ml^{-1}$)				
Baseline	19.9 ± 2.8	19.2 ± 1.0	14.3 ± 1.6	*0- & 2-Hr > 4-Hr*
Final	15.3 ± 2.0	15.6 ± 1.5	8.8 ± 0.6	*0- & 2-Hr > 4-Hr*
P	*0.09*	*0.03*	*0.001*	
FVIIa ($ng \cdot ml^{-1}$)				
Baseline	3.64 ± 0.13	4.23 ± 0.28	3.80 ± 0.23	*0- & 4-Hr < 2-Hr*
Final	3.40 ± 0.18	2.88 ± 0.03	3.10 ± 0.07	*0- & 4-Hr > 2-Hr*
P	*0.18*	*0.0002*	*0.004*	
TFPI/Xa ($nmol \cdot L^{-1}$)				
Baseline	0.35 ± 0.05	0.36 ± 0.05	0.34 ± 0.05	*No sig. dif.*
Final	0.37 ± 0.04	0.41 ± 0.04	0.40 ± 0.04	*0-Hr < 2- & 4-Hr*
P	*0.17*	*0.02*	*0.002*	

Data are means ± SE (n=8); Test conditions: Baseline (before aerobic exercise training), Final (after 6-months of aerobic exercise training); PAI-1: plasminogen activator inhibitor-1; FVIIa: plasma activated factor VII; TFPI/Xa: Tissue factor pathway inhibitor-activated factor X complex. P represents mean comparisons between tests at each level of time (i.e. fasting baseline vs. fasting final). Mean differences within test represents a significant difference between time points within a single test (i.e. fasting baseline vs. 2-hr baseline vs. 4-hr baseline).

Appendix F: Figures

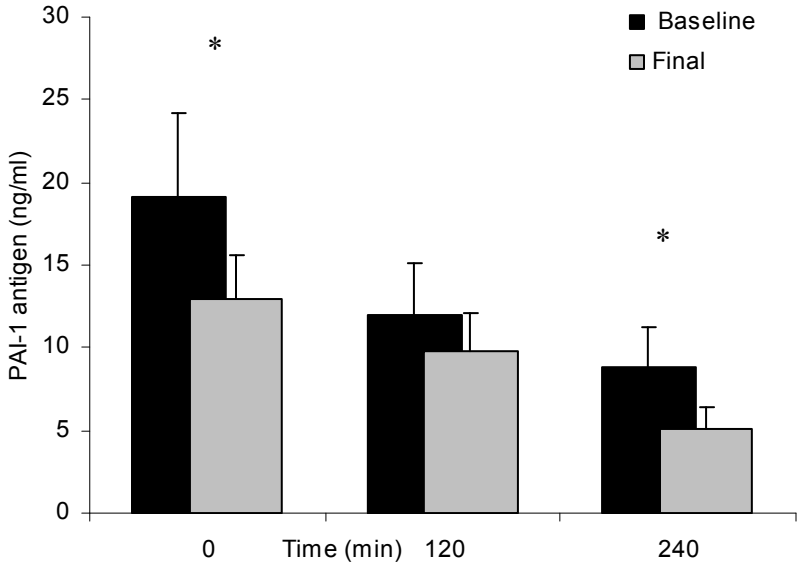
Figure 1. Average CHO and Fat oxidation during the entire 4-hour lipemia test before and after exercise training.



Data are means ± SE (n=6); Baseline (before aerobic exercise training), Final (after 6-months of aerobic exercise training); CHO: Estimated total carbohydrate oxidation rate; Fat: Estimated total fat oxidation rate.

*Means are significantly different between tests within a specific timepoint. Please refer to table 4 for the differences between time-points.

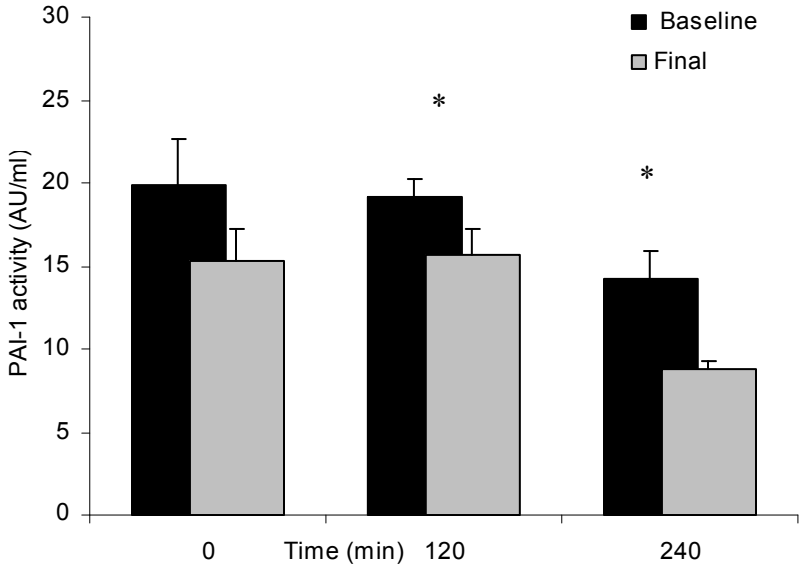
Figure 2: Fasting and 4-hour postprandial PAI-1 antigen before and after exercise training.



Data are means ± SE (n=8); Baseline (before aerobic exercise training), Final (after 6-months of aerobic exercise training); Time: Minutes; PAI-1 antigen: plasminogen activator inhibitor-1 antigen.

*Means are significantly different between tests within a specific timepoint. Please refer to table 4 for the differences between time-points.

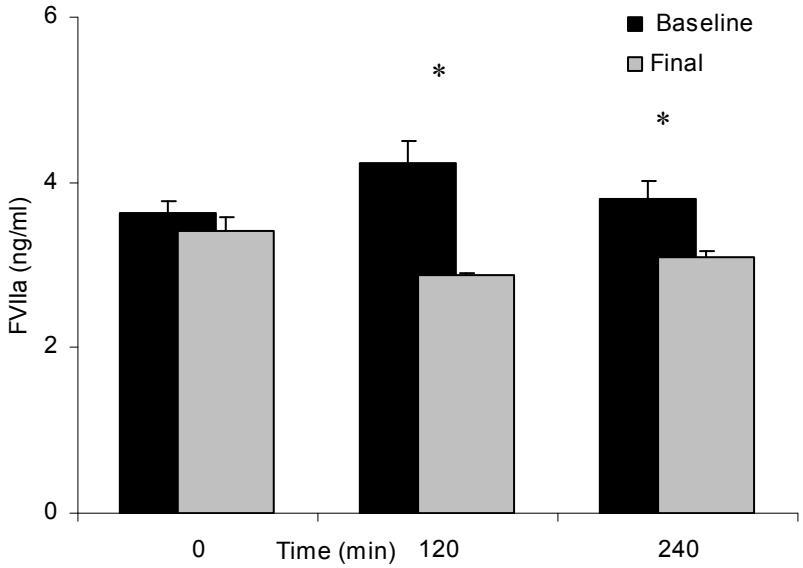
Figure 3: Fasting and 4-hour postprandial PAI-1 activity before and after exercise training.



Data are means ± SE (n=8); Baseline (before aerobic exercise training), Final (after 6-months of aerobic exercise training); Time: Minutes; PAI-1 activity: plasminogen activator inhibitor-1 activity.

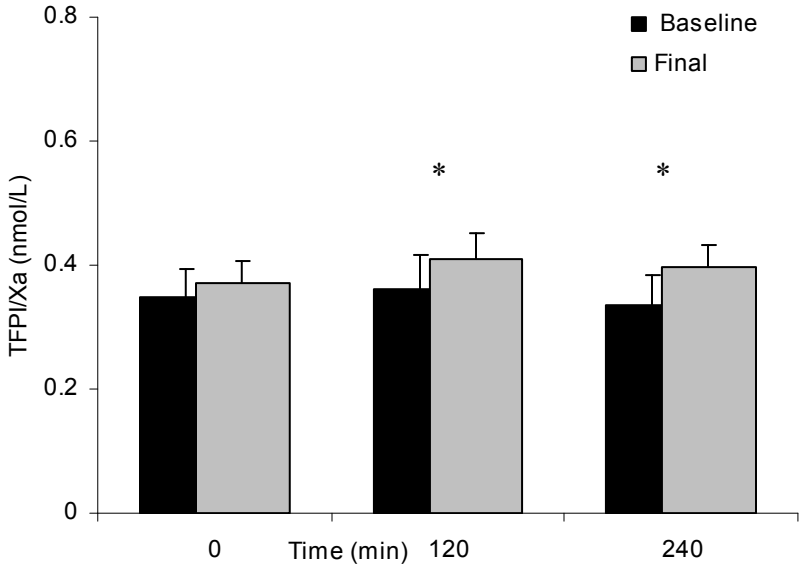
*Means are significantly different between tests within a specific timepoint. Please refer to table 4 for the differences between time-points.

Figure 4: Fasting and 4-hour postprandial FVII activity before and after exercise training.



Data are means ± SE (n=8); Baseline (before aerobic exercise training), Final (after 6-months of aerobic exercise training); Time: Minutes; FVII: plasma factor VII. *Means are significantly different between tests within a specific timepoint. Please refer to table 4 for the differences between time-points.

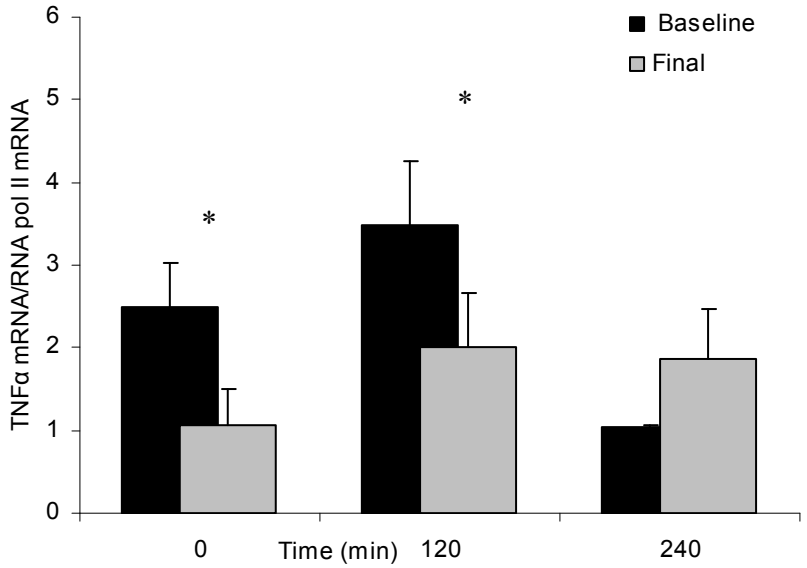
Figure 5: Fasting and 4-hour postprandial Tissue factor pathway inhibitor/factor Xa complex concentrations before and after exercise training.



Data are means ± SE (n=8); Baseline (before aerobic exercise training), Final (after 6-months of aerobic exercise training); Time: Minutes; TFPI/Xa: Tissue factor pathway inhibitor- Activated plasma factor X complex:.

*Means are significantly different between tests within a specific timepoint. Please refer to table 4 for the differences between time-points.

Figure 6: Fasting and 4-hour postprandial relative TNF-α gene expression before and after exercise training.



Data are means ± SE (n=4 at 0- and 2-hr and n=3 at 4-hr); Baseline (before aerobic exercise training), Final (after 6-months of aerobic exercise training); Time: Minutes; TNF-α: Tumor necrosis factor alpha; mRNA: messenger RNA; RNA pol II: RNA polymerase II.

*Means are significantly different between tests within a specific timepoint. Please refer to table 4 for the differences between time-points.

Appendix G: Review of Literature

Ingestion of a high fat meal

Following the ingestion of a high-fat meal, the food enters the alimentary canal, where it is then deposited in the stomach. Very little fat digestion occurs in the stomach; however gastric lipase hydrolyzes one out of every four triglyceride molecules in the stomach (55). The majority of fat digestion occurs in the upper two-thirds on the intestine (duodenum and jejenum) and this process is mediated by several hormones as well as nervous system innervation. The linguinal and gastric glands have very little impact on the digestion process of fats, with the exception of altering pH. When a bolus of food enters the stomach, the proteins are digested by enzymes that function in a low pH optimum. The acidic contents of the gastric chamber will damage the enterocytes lining the small intestine, and therefore must be neutralized prior to entering the duodenum (55).

The chemoreceptors within the intestine are sensitive to fat which signals the release of CCK into the blood. This hormone slows gastric emptying, as fat digestion is a much slower process than that of carbohydrate and protein digestion. This also allows more time to neutralize the contents of the stomach as it enters the duodenum (56). The bolus of gastric content that is released into the intestine is known as chyme. Chyme is a hyper-osmotic and acidic solution that requires bicarbonate (HCO_3^-) in order to be neutralized. In addition to slowing gastric emptying, CCK also induces the release of HCO_3^- from the pancreas. Osmoreceptors in the intestinal wall are sensitive to the osmolarity of the chyme, and when the osmolarity is high,

they further inhibit gastric emptying. As the HCO_3^- neutralizes the intestinal contents, gastric emptying increases and the chyme is then able to undergo duodenal digestion (56).

Fats are insoluble in aqueous solutions, and therefore the triglycerides (TG) in the high-fat meal must undergo emulsification prior to enzymatic degradation (57). Short (C:2 – C:4) and medium (C:6 – C:12) chain TG are much less hydrophobic than long (> C:14) chain TG, and are able to bypass the processes of emulsification, degradation, re-esterification, and chylomicron formation that are outlined below (57). Instead, short and medium chain TG are able to be rapidly absorbed in the luminal surface and enter directly into the portal circulation bound to plasma albumin. Long chain TG are much more hydrophobic and are transported in plasma within chylomicrons (57). Chylomicrons are too large to enter the capillaries, therefore they enter the intracellular space via exocytosis, collect in the lacteals, and travel toward the thoracic venous system via the lymphatic vasculature. The chylomicrons finally enter the circulation in the upper vena cava.

The first step in the digestion of ingested fats is the emulsification process, mediated by bile acids that are stored and released from the gall bladder and liver (58). The stimulus for the release of bile is mediated by CCK, which induces the relaxation of the sphincter of Oddi and contraction of the gall bladder (56). This allows bile to enter the common bile duct and subsequently flow into the duodenum.

Large micelles are formed by the ingested TG and free fatty acids hydrolyzed by gastric lipase. Once in the intestine, bile salts bind to the surface of the large micelles, due to its amphipathic structure. Bile is synthesized from cholesterol and

contains lecithin and bilirubin. The polar head of the bile salt is exposed to the aqueous solution while the hydrophobic section interacts with the lipids in the large micelle. In this process, the hydrophobic TG molecules are separated from water in the intestine and the polar surface allows it to remain in solution (57).

Following emulsification by bile salts, the ingested fat is now able to undergo digestion. This process is mediated by pancreatic lipase and colipase, both of which are secreted from the pancreas. Pancreatic lipase is structurally and functionally similar to other lipases, such as lipoprotein, endothelial, and hepatic lipases, but not gastric or hormone-sensitive lipase (59). A key element to the function of the former group of lipases is the fact that they are anchored to the cell surface, and this function is achieved via colipase on the surface of the emulsified lipids. Additionally, pancreatic lipase is secreted from the pancreas in a folded, inactive state. This prevents the enzyme from premature activation and subsequent degradation of pancreatic cell membrane acyl-glycerols. When pancreatic lipase binds to the surface of the emulsified lipid in the intestine, the presence of colipase anchors it to the surface and unfolds the enzyme, thus exposing the active site (59).

Pancreatic lipase has specific activity toward the sn1 and sn3 ester bonds of the TG. The typical pattern of FFA hydrolysis begins at the sn1 position, which increases the activity of pancreatic lipase for the sn3 position (59). This process results in the formation of 1 R_2 monoacylglycerol (MAG) and two FFA for every one TG. The amount of pancreatic lipase secreted is 100-1000 times in excess of that which is needed for complete hydrolysis of the ingested fat. Thus, nearly complete

intestinal absorption of TG is achieved, with less than 5% of the total content being lost to fecal material (59).

The liberation of FFA and the formation of MAG allows the size of the emulsified micelles to be reduced. The smaller micelles are able to migrate to the brush border of the proximal jejenum where they encounter a thin layer of water covering the luminal surface of the enterocytes, known as the unstirred water layer (57). This serves as a barrier to the lipids, and this is overcome by the bile salts through disruption of hydrogen bonds of the water. Within this process, the low pH of the unstirred water layer serves to disperse and disrupt the micellar-bile bonds and liberate the FFA and MAG. Through passive diffusion, the ingested lipids are then able to enter the enterocyte for re-esterification and packaging into chylomicrons (57). The liberated bile salts are then reabsorbed in the ileum, enter the portal circulation, are recycled by hepatocytes and stored in the gall bladder for later release.

Once taken up by the enterocytes, the FFA bind to cytosolic fatty acid binding protein (FABP) in order to prevent the cytotoxic effect and diffuse through the cytosol to the luminal surface of the smooth endoplasmic reticulum (SER) (60). Once inside the SER, the re-synthesis of TG occurs, primarily by the sn2-MAG pathway (80%), and the remaining 20% of FFA are synthesized into phospholipids (60). For the purpose of this outline, only the former will be discussed, as the latter pathway has little relevance to cardiovascular disease.

The enzymes of TG synthesis in the enterocyte SER are bound to the inner membrane. The first enzymatic activity in the re-esterification process within the SER is that of acyl-CoA synthetase, which activates the fatty acyl via ATP and

coenzyme A (60). The products are a free fatty acyl-CoA that is now activated for esterification, and AMP + $2PP_i$. There are specific acyl-CoA synthetases for fatty acids of different chain lengths, although the reaction mechanisms are the same for all isozymes. The next step in TG synthesis is mediated by monoacylglycerol acyltransferase, and it has preferential activity toward sn2 MAG. It transfers a free fatty acyl-CoA to the sn1 position of a MAG, with sn1-sn2 diacylglycerol (DAG) as the product of the reaction. The initial acyl-CoA synthetase reaction must occur twice during the formation of a TG from a MAG, and the product of the second reaction is transferred to the DAG via DAG-acyltrasferase in a mechanism similar to that of MAG trasnferase, with the exception that it esterifies the activated fatty acyl-CoA in the sn3 position. The net of the three reactions outlined above is the formation of one TG at the cost of 4 ATP (60).

The formation of TG within the SER results in the accumulation of fat droplets inside the lumina, which are collected into membrane vesicles. The accumulated TG can not leave the SER independently, as they are extremely hydrophobic and can not enter the cytosol or the plasma. Thus, TG are packaged in chylomicrons (CM) inside the SER. CM are large particles containing lipoproteins, cholesterol, TG, and minor amounts of FFA. The majority of lipoproteins are apolipoprotein B-48 (ApoB-48), A-I (ApoA-I), A-II (ApoA-II), and A-IV (ApoA-IV), with lower amounts of ApoC and ApoE. ApoB-48 is the major structural protein of the CM and serves as the TG binding portion of the pseudo-micelle (61;62). ApoA-I and ApoA-II enter the circulation bound to CM, however they are rapidly transferred to nascent high density lipoproteins (HDL), in a reverse cholesterol

transport (RCT) mediated process. ApoA-IV is only synthesized in the intestine unlike most other apolipoproteins that are of both hepatic and enterocyte origin, and its role in CM biology is unknown (61;62). ApoC-II, ApoC-III, and ApoE are known to be incorporated within the CM structure; however they are not found on CM within the enterocytes. It is likely that these apolipoproteins are transferred to the CM in plasma during RCT mediated processes. Their role in CM metabolism, which will be discussed in detail below, is to regulate the rate of lipoprotein lipase activity.

The first step in the synthesis of CM is the secretion of ApoB-48 in the enterocyte (61;62). While the complete mechanisms underlying this process have not been fully elucidated, several of the steps have been shown to be similar between hepatocytes and enterocytes. The main difference between the pathways in these two tissues is that the ApoB protein is truncated in the intestine, such that only 48% of the original peptide is synthesized. The ApoB-48 protein is translated on the surface of the rough endoplasmic reticulum (RER) by ribosomes, after which it is translocated into the lumen of the SER and resides in the membrane (61;62). There is direct competition between the rate of degradation and the rate of translocation, which is dependent on the supply of TG. Thus, in the fasted state, it appears that ApoB-48 degradation would exceed that of translocation. However, following a high-fat meal, ApoB-48 translocation to the SER membrane would occur much faster and exceed its rate of degradation.

It has been proposed that during ApoB-48 translation, small amounts of lipid particles interact with and bind to the hydrophobic residues of the emerging protein. This process ensures that proper folding of the tertiary structure occurs by preventing

the lipophilic domains from achieving non-specific interactions with themselves, other proteins, or membrane phospholipids (61;62). The result of this process is the formation of a small, dense emulsion particle that is capable of undergoing further lipidation post-translationally in a two-step process of CM synthesis (61;62).

After formation of the emulsion particle, the total lipid content of the nascent ApoB molecule in this phase is insufficient to overcome the rate of pre-secretory degradation. In order to rapidly and efficiently promote lipid accumulation of ApoB during CM synthesis, the dedicated, SER-localized, cofactor microsomal triglyceride transfer protein (MTP) is required (63-65). During translocation from the RER to the SER, ApoB-48 acquires lipids and cholesterol from membrane vesicles in the lumen of the SER, and the secretory route enables the nascent ApoB molecule to interact with MTP, which mediates the transfer of TG, phospholipids, and cholesterol from membrane vesicles and the ER lumen to the core of the folded ApoB-48 molecule (63-65). This process ensures complete lipidation and core expansion of the nascent CM particle.

The next step in the synthesis of CM is transport to the golgi apparatus for pre-secretory modification through increased phosphatidylcholine (PC) content and apolipoprotein glycosylation (66). There appears to be a sufficient amount of phospholipid (PL) in the pre-secretory form of CM, however the golgi apparatus increases the relative amount of PC from 50 to 80% of the total PL content (66). It is unknown how the golgi modifications affect the function of CM in peripheral tissues, as very little data exists on the functional aspects of pre-golgi versus post-golgi modified CM. It is known that the unmodified form is capable of exiting the cytosol,

although this form has not been observed in lymph or plasma under normal conditions.

Glycosylation is the process of adding a glycosyl group, derived from the cyclic form of glucose, after removal of the hemiacetal hydroxyl group via formation of a Schiff's base intermediate, to a functional protein. This process alters the quaternary structure of a protein, and it has been shown to enhance cellular recognition of the ApoA-I and ApoB-48 particles (67). The amount of glycated end-products in lipoproteins has been shown to mediate the atherogenic nature of remnant lipoproteins following delipidation in peripheral tissues (67). Furthermore, advanced glycosylation end-products (AGE) are known to exist in diabetic states and trap lipoproteins within the intimal layer, prevent the release of nitric oxide with concomitant expression of reactive oxygen species, and promote inflammation and monocyte recruitment (67).

Following glycosylation in the golgi apparatus, the CM is now capable of being released from the enterocyte via exocytosis into the intracellular space. This process occurs when the golgi vesicles containing the CM migrate toward the lateral plasma membrane and fuse to the inner membrane (66). It is not known how the golgi vesicles open following attachment to the plasma membrane, or what the fate of the golgi complex is following exocytosis, although ample data is available in support of this process.

The CM are released into the basolateral space between the enterocytes, and travels to the lamina propria (LP) (68). CM are too large to enter the plasma circulation through the leaky junctions of capillary endothelial cells, thus transport

must begin within the lymphatic circulation. The LP contains vessels that collect in the lacteals which transport the lymph from the mesenteric vessels to the thoracic duct. The transport distance from the intracellular space to the lacteals is approximately 50μm, and there are two possible mechanisms responsible for CM movement (68). One is through diffusion, and another is through fluid movement caused by connective tissue electrolyte exchange. In either case, the rate of lymph flow directly affects the rate of appearance of CM in the lacteal collecting ducts.

Following CM entry into the lymphatic vessels, it appears in the plasma circulation within the superior vena cava. The TG contained within lipid rich CM are not metabolized by hepatocytes, rather they are delivered to peripheral tissues where the activity of lipoprotein lipase (LPL) hydrolyzes the ester bonds between the acyl groups and the glycerol backbone. The mechanisms and exercise training adaptations of TG and FFA metabolism are described in detail in section 3, although it is necessary to describe the mechanisms for transport and delivery to the peripheral tissues prior to any discussion of metabolism. Thus, for the remainder of this discussion, the focus will remain on hepatic clearance of remnant CM particles (RCMP).

Chylomicron metabolism

Fatty acids are typically carried in plasma in the form of triacylglycerols (TG) in complex with chylomicrons and/or very low density lipoproteins (VLDL). Some free fatty acids (FFA) are carried in plasma bound to albumin, however the majority is in the form of TG in complex with chylomicrons and/or VLDL (69). The rate of TG uptake is due in large part on the plasma concentration (69); however there are

several components that contribute to clearance in addition to this. These additional factors can be divided into two main categories: One is the lipolytic capacity of the tissue, and the second is the storage capacity of the tissue.

There are several metabolic enzymes that contribute to the lipolytic capacity of the tissue of interest, in which all contribute to the clearance rate of plasma TG. The first to be discussed is lipoprotein lipase (LPL). LPL is a proteoglycan linked enzyme that resides on the luminal surface of endothelial cells. It is the enzyme responsible for the majority of TG hydrolysis from cholesterol (chylomicrons and VLDL), and it is expressed in skeletal and cardiac muscle, adipose tissue, and monocytes (70-72). There are two main pools of LPL, one is the heparin releasable portion that is bound to the endothelial cell surface, and the other is the inducible fraction that is stored within sarcoplasmic reticulum in skeletal and cardiac muscle (71;72).

The apolipoprotein content of cholesterol is widely held to be the determinant of specificity for substrate recognition, and it is believed that LPL has specific activity toward ApoB-100 and ApoB-48 containing lipoproteins (71;72). ApoB-100 is expressed predominantly in hepatic-derived cholesterol fractions (VLDL) and ApoB-48 is expressed in intestinal-derived cholesterol fractions (chylomicrons), both of which are responsible for the amount of TG contained in the cholesterol particle by serving as the TG emulsifying portion. Chylomicrons also contain ApoC-II and ApoC-III, of which the former activates and the latter inhibits LPL activity. ApoC-II is the apolipoprotein that activates TG hydrolysis into free fatty acids within the

capillary endothelium. The released FFA is now able to enter the tissue were it can be esterified and enter into the mitochondrial β-oxidation pathway.

Following ingestion of a high fat meal, the intestinal epithelium increases the rate of ApoB-48 expression, which increases the size as well as the total concentration of chylomicrons secreted into the lymphatic system (65;69). Chylomicrons then enter the plasma circulation by way of the superior vena cava, after which LPL is then capable of initiating TG hydrolysis (69). When the plasma chylomicron concentration increases, LPL activity increases in muscle, liver and adipose tissue. However, the rate at which LPL hydrolysis occurs is dependent, in part, on the tissue's energy stores and metabolic demands (70).

Following LPL-mediated TG hydrolysis, FFA are released from chylomicrons and/or VLDL and diffuse to the cellular membrane. Initially, it was believed that FFA were able to passively diffuse through the cellular membrane of peripheral tissues, however it has been noted that this effect reaches a saturation point in vivo (55;57;58;73). Thus, it was determined that membrane transport and carrier proteins are responsible for the majority of FFA uptake into the tissues.

There are three main proteins that contribute to FFA flux across the cellular membrane. The first is the plasma membrane fatty acid binding protein (FABP(PM)), and it is expressed in cardiac and skeletal muscle (74). It can be activated via contraction-mediated translocation, and its sarcolemmal translocation pattern is similar to that of GLUT4. This protein is responsible for the carrier-mediated transport of long-chain fatty acids (fatty acids > 14- to 18-carbons), while fatty acid

transport protein-1 (FATP-1) is responsible for the transport of very-long chain fatty acids (>18-carbons) across the plasma membrane (75).

The third protein involved in FFA transport across the sarcolemmal is fatty acid translocase (FAT/CD36). Similar to FABP(PM), FAT/CD36 expression is increased following muscular contraction and insulin mediated 2^{nd} messenger signaling pathways (76). However, the exact second messengers have not been elucidated. It appears that peroxisome proliferator activated receptors-alpha and gamma (PPAR-α PPAR-γ) are capable of increasing FAT/CD36 expression in hepatic tissues but not in skeletal or cardiac muscle (76). In addition to its expression on the sarcolemma, it is also expressed on the mitochondrial membrane and a novel function in the regulation of β-oxidation has been suggested (76).

It is now thought that fatty acid translocase is capable of regulating the activity of carnitine acyl transferase-1 (CAT1) (77). CAT1 is the enzyme responsible for transporting activated fatty acids (fatty acyl-CoA) from the cytosol into the mitochondria, and it is a major site of regulation in the β-oxidation pathway (78). Thus, increasing the activity of CAT1 will increase the flux of FFA through metabolism and increasing the clearance of plasma TG levels (77;78). CAT1 catalyzes the transfer of the thiol-ester (CO-S) bond of the acyl-coenzyme-A (CoA-SH) to carnitine, which allows the fatty acyl-carnitine to cross the inner-mitochondrial membrane. Once inside the mitochondria, carnitine acyl transferase-2 catalyzes the transfer of the thiol-ester bond of the fatty acyl-carnitine to CoA-SH, thereby reforming the activated acyl-CoA inside the mitochondrial matrix (78).

Several factors are responsible for the regulation of CAT1, all of which have a direct influence on the flux of cytosolic FFA; however the main regulators of CAT1 activity are malonyl-CoA and citrate (77). Malonyl-CoA is formed by the addition of bicarbonate (HCO_3^-) to acetyl-CoA via the enzyme acetyl-CoA carboxylase in an ATP dependent manner. In the presence of increased malonyl-CoA concentrations, CAT1 activity decreases (77;78).

Acetyl-CoA carboxylase is inhibited by phosphorylation via protein kinase A (PKA) or increased cytosolic FFA concentrations (79). Inhibition is mediated by promoting the precipitation of the phosphorylated form of the enzyme out of active polymers. Activation of acetyl-CoA carboxylase is mediated by increased cytosolic citrate concentrations (79). Citrate is a potent allosteric activator, and after binding to the acetyl-CoA carboxylase regulatory sites, it promotes polymerization of the enzyme into its active form, in spite of phosphorylation of the inhibitory sites. Finally, the effect of citrate is easily diminished in the presence of low concentrations of acyl-CoA, although this effect is negated when PKA phosphorylation of acetyl-CoA carboxylase is not present (79).

Following CAT2-mediated reformation of acyl-CoA in the mitochondrial matrix, the substrate enters into the β-oxidation pathway. Large amounts of ATP and reduced coenzymes are produced from a single fatty acyl-CoA, of which there are three for every TG molecule. At rest, the energy released rapidly exceeds demand, and the majority of ingested FFA is channeled toward storage as intramuscular TG droplets or as TG in adipose tissue.

As stated above, there are two factors that contribute to the reduction in plasma TG following a high fat meal. The first, which was discussed above, is lipolytic capacity, and the second is the storage capacity of the tissue. Storage capacity refers to the tissue's ability to uptake TG, and with the exception of adipose tissue, it is determined in large part by cellular energy demands. Skeletal and cardiac muscle have the capacity to store TG within the intracellular vacuoles; however the relative amount of intramuscular TG is much lower than adipose tissue (80;81).

In addition to the fact that myocellular storage capacity is low, the energy demand of skeletal muscle during rest is low. During physical activity, energy demand increases over 10-fold from resting conditions and the rate of TG uptake increases to meet this demand. However, at rest, a high fat meal combined with glucose will cause the majority of plasma TG to be directed to white adipose tissue for storage (82). The low-level energy demands at rest are met by glycolytic metabolism due to the rise in insulin concentrations that occur concomitantly with the ingested glucose.

The effect of increased β-oxidation approximately two-hours postprandially is mediated in the same manner as that during fasting conditions. The inhibition of skeletal and cardiac muscle LPL is removed, which allows for increased chylomicron TG hydrolysis (72). In the absence of physical activity, energy demands are low, and the majority of plasma TG continues to be directed toward adipose tissue storage, in spite of the fact that glucose levels have decreased. This illustrates the necessity for greater caloric expenditure following the consumption of a high fat meal. If energy

demands are increased, more TG will be consumed via β-oxidation and the amount of TG stored will be reduced.

Triglyceride and glucose metabolism

In the fasted state, prior to meal ingestion, insulin concentrations are low and glucagon levels are high, compared to the postprandial state. The effect of these hormones on lipid metabolism causes an increase in fat oxidation and glucose/glycogen sparing (83). This effect is mediated through covalent and allosteric regulation of several metabolic enzymes, one of which is hormone sensitive lipase (HSL). HSL is expressed in adipose tissue, skeletal muscle, and it is believed to be active in cardiac muscle, although in lower quantities. HSL is activated by glucagon and other cyclic-AMP activating agents and it is inhibited by insulin (84).

LPL in adipose tissue functions in a reciprocal manner counteracting the effect of HSL. LPL serves to promote TG storage within the adipocyte and HSL serves to promote the release of FFA and glycerol into the circulation. In skeletal and cardiac muscle, these two enzymes work in concert to promote FFA esterification and lipid oxidation (84). LPL releases FFA from chylomicrons and VLDL, and HSL promotes the esterification of stored lipid droplets within the vacuoles, releasing intracellular FFA into the cytosol. In adipose tissue, these two enzymes regulate the rate of storage and release, and in skeletal and cardiac muscle, they regulate the flux of FFA into β-oxidation (84).

The ingestion of a high fat meal combined with glucose will cause an increase in insulin and a decrease in glucagon, compared to the fasting state. The effect of this on lipid metabolism will be to decrease the rate of lipid oxidation in muscle and

increase the rate of storage in adipose tissue (83), plasma TG will decrease, and there will be a shift in tissue sensitivity. Insulin will inhibit HSL and increase the activity of LPL in muscle and adipose tissue. Substrate utilization will begin to favor glucose metabolism and TG will be stored predominantly in adipose tissue until insulin levels return to normal.

This process is achieved via reciprocal regulation of several metabolic enzymes, the first of which is the activation of insulin receptor substrate-1 (IRS-1). The tyrosine kinase activity of the insulin receptor activates IRS-1 which then phosphorylates and activates phosphatidylinositol-3-kinase (PI3K). PI3K then converts phosphatidylinositol-3,4-bisphosphate into phosphatidylinositol-3,4,5-triphosphate (PIP_3). PIP_3 activates phosphatidylinositol-dependent kinase-1 (PDK1) which then initiates a wave of phosphorylations which results in an increase in the rate of glycogen synthesis (85).

In addition to activating glycogen synthesis in muscle and liver, insulin-stimulated PDK1 activation phosphorylates phosphoprotein phosphatase-1 which dephosphorylates and deactivates HSL. The result is a decrease in TG release and an increase in TG uptake in adipose tissue. This process continues in the presence of increased plasma insulin levels and following the return of glucose to normal levels, skeletal muscle TG uptake and β-oxidation will again increase.

The rise in plasma insulin levels following a single meal is transient, and is expected to disappear within one- to two-hours. After this time, it is reasonable to assume that the body has achieved homeostasis with respect to plasma glucose levels. It is at this point that the inhibition of lipolysis mediated by insulin is diminished to

the point that cellular energy demands are once again met by plasma TG. This has been observed in our laboratory, as the respiratory exchange ratio consistently increases from baseline (0-hour) to 2-hours postprandial, and then decreases from 2-hours to 4-hours postprandial (unpublished data). These results confirm the fact that β-oxidation decreases following the ingestion of a fat meal during the time that insulin levels are elevated, and then increases following glucose homeostasis.

We have also found that the RER is altered in individuals following a high fat meal after exercise training (unpublished data). The estimated rate of β-oxidation is higher 2-hours after a meal (lower VCO_2/VO_2), which suggests that the rate of lipid metabolism is higher following training. There are several reports that either directly or indirectly confirm this observation, which is likely due to the fact that physical activity increases the metabolic and storage capacity of skeletal muscle (86-90). Furthermore, it is well known that insulin sensitivity and the rate of glucose disposal is increased with training (83;89).

The metabolic capacity of skeletal muscle increases due to an increase in LPL expression, FFA transport protein density, mitochondrial density, and oxidative enzyme content. LPL expression increases due to an increase in muscle capillary density, which provides for a greater capacity for chylomicron and VLDL TG hydrolysis. Second, there is an increase in fatty acid translocase (FAT/CD36) and plasma membrane fatty acid binding protein expression (76). This effect is mediated in two ways, the first of which is an increase in gene expression and the second is an increase in contraction-mediated translocation of FAT/CD36 to the sarcolemmal membrane, similar to that of GLUT4 (76). When the effect of increased LPL

expression is combined with an increase in FFA transport proteins, the overall effect is to significantly enhance the capacity for FFA delivery to the muscle.

In addition to the transport capacity, the lipolytic activity of skeletal muscle is greater following exercise training (71;72). This allows for a greater capacity of FFA flux through β-oxidation at rest. Several of the regulatory oxidative enzymes in skeletal muscle have been shown to have higher concentrations after exercise training. The elevated metabolic demand of physical activity causes CAT1, pyruvate dehydrogenase complex, and citrate synthase (as well as many others) gene expression to increase, all of which increase oxidative metabolism capacity. It is likely that energy demand during exercise depletes myocellular glycogen and lipid stores, and a greater capacity for substrate entry into the tissue is needed in order to replenish energy stores.

In addition to gene expression, the lipolytic capacity is increased after exercise training due to an increase in insulin sensitivity. GLUT4 concentration and density is higher after training (91;92), and this effect is mediated in two ways. The first is through contraction-induced migration to the sarcolemma, where it can become functional at a lower activation threshold. Second, GLUT4 gene expression increases, which increases both the sarcolemmal density and the concentration of the inducible pools within the cytoplasm (91;92). Thus, the capacity to uptake and clear circulating plasma glucose is much greater in the trained state relative to the sedentary state.

If the ability to clear plasma glucose is higher after exercise training, then it is logical to assume that insulin-stimulated glucose disposal will increase, and the

concentration of insulin will decrease. This is what is meant by an increase in insulin sensitivity. Less insulin is needed in the trained state compared to the sedentary state to have the same rate of glucose disposal. Increased insulin sensitivity will decrease postprandial insulin concentrations, and the inhibition of lipolysis will be diminished or even abolished after exercise training.

The effect of increased lipolytic capacity after training due to decreased postprandial insulin concentrations would occur independently of the intensity of training. Prolonged physical activity will deplete glycogen stores at higher intensities (>60% VO_2max), and metabolic demand will be met through stored and plasma TG at lower intensities. In either case, contraction-mediated GLUT4 activity will be higher following exercise training, independent of intensity, and dependent on total energy expenditure.

It has been consistently shown that β-oxidation increases in the recovery period after an acute bout of exercise, and this is believed to be due to glucose/glycogen sparing in hepatic tissue and skeletal muscle (87;88;90). In order to explain this observation in light of the fact that GLUT4 expression and sensitivity is higher after an acute bout of exercise, it was determined that glucose-6-phospahte is directed toward glycogen synthase through a decrease in hexokinase activity (89). If glycogen synthesis increases, then β-oxidation is the most likely method for meeting the energy demand of basal metabolism following exercise.

In summary, the addition of insulin to a single high fat meal will decrease the rate of TG uptake due to insulin-mediated inhibition of lipolytic enzymes. This effect is transient, and it is believed to disappear within two hours postprandially, at which

point TG clearance increases. Following exercise training, metabolic capacity and insulin sensitivity is higher, and the inhibition of lipolysis is expected to be lower. This would allow for a greater flux of FFA through β-oxidation, and the clearance of plasma TG would occur much sooner after training.

Chylomicron remnant clearance

The clearance of remnant chylomicron particles (RCMP) from circulation is believed to occur in three steps, the first of which is uptake into the space of Disse through the fenestrated endothelium (93). The openings in this space are too small to allow large CM to pass, which prevents hepatic clearance of TG-rich CM. However, the smaller RCMP are able to enter the space of Disse where large amounts of heparin sulfate proteoglycans (HSPG) and ApoE are present (93). The HSPG serve as receptors that bind and localize ApoE within the space, as well as to trap RCMP that have entered.

Receptor-mediated hepatic endocytosis of RCMP may occur in any of three fashions: 1) either through HSPG independent endocytosis, 2) low density lipoprotein related receptor (LRP) bound HSPG, or 3) low density lipoprotein receptor (LDLR) independent of HSPG (93;94). In each of the three mechanisms listed above, there is a common factor in which RCMP bind to the receptor(s) which are then internalized via endocytosis, subjected to ubiquination, channeled to clathrin-coated pits, and subsequently degraded in lysosomes or recycled. The detailed mechanisms of this process will be discussed below.

ApoE and ApoB-48 each have heparin binding domains within the protein sequence and are known to be sequestered in vivo by HSPG (93). This may serve as

the initial step in removing RCMP from the circulation, after which further lipolytic degradation via HL and LPL reduces the size and TG content of the CM. In addition to this process, the relative cholesterol content of the remnant increases, and the ApoE expressed on the surface can interact with the LDLR or LRP (93;94). In the absence of LDLR or LRP, HSPG are capable of internalizing following ligand binding, however this process is much slower than that of LDLR and LRP internalization.

The LDLR shares over 50% sequence homology with the LRP and each receptor has HSPG and ApoE binding domains (94). It has been proposed that fractions of circulating CM are complexed with LPL in plasma, which further facilitates HSPG/LDLR or HSPG/LRP binding to the RCMP (94). The functionality of this process has been questioned due to an underwhelming amount of knowledge as to the contribution of plasma LPL to CM metabolism. Studies in which human LPL was over-expressed in hyperlipidemic and hypercholesterolemic rabbits reduced the levels of circulating remnant particles, however the amount of infused LPL greatly exceeded that which is seen under physiological conditions (95;96).

The most compelling evidence for the contribution of LRP and LDLR in the clearance of RCMP comes from knock out (KO) animal models. LDLR KO models have consistently shown that absence of the receptor induces severe hyperlipidemia and hypercholesterolemia (97-99). Complete removal of the LRP gene causes embryos to die in utero, however this aspect was overcome by co-expression of the α_2-Macroglobulin receptor associated protein (RAP) gene. RAP binds to the LRP and inhibits its activity, thus rendering it ineffective; RAP also binds to the LDLR

although with a much lower affinity (94;100). The level of hypercholesterolemia in the RAP expression animal models was the same as that which was observed in the LDLR KO animal models (94). In each of the models listed above, the cause of the increased plasma lipoprotein levels was attributed to deficiencies in hepatic remnant particle endocytosis.

Following ligand binding to the respective receptor (i.e. HSPG, LDLR, or LRP), it is internalized and targeted for lysosomal degradation (101). A drop in the pH within the lysosome causes the cholesterol to dissociate from the lipoproteins of the CM, and the cholesterol is either recycled or degraded. If it is recycled, it can be used in either very low density lipoprotein biosynthesis, integrated into the membrane, or used for hormone synthesis in other tissues. The TG and protein components of the RCMP are degraded in the lysosome and targeted for catabolic or biosynthetic pathways (102). Thus, receptor-mediated endocytosis efficiently removes remnant lipoproteins from the plasma, with the majority of this activity occurring in the hepatic tissue.

Hemostasis

Historically, the process of coagulation has been referred to as a cascade of enzymatic reactions that includes intrinsic and extrinsic pathways. However, the terms can be misleading. A cascade implies a step-wise process leading from one reaction to another, and the process of coagulation involves many positive and negative feedback mechanisms through activation of plasma proteins. It is therefore, more accurate to refer to coagulation as a pathway than a cascade.

As mentioned above, coagulation involves the intrinsic and extrinsic pathways. The proteins involved in the activation of the intrinsic and extrinsic pathways differ, but they both lead to the same result: factor X activation and subsequent thrombin and fibrin formation. The intrinsic pathway is activated when elements of the coagulation pathway come into contact with a negatively charged surface. The extrinsic pathway is activated when factor VII is exposed to tissue factor expressed on disrupted, nucleated cell membranes. The intrinsic pathway includes all of the coagulation proteins present in plasma that are required for the formation of thrombin as well as an anionic (negatively charged) surface that is not present in plasma, which initiates contact activation (103).

Five factors are required to initiate contact activation within the intrinsic pathway of coagulation. The first is factor XII, and it is a plasma glycoprotein that circulates in an inactive form. In its active form it is a serine protease, and normal human plasma contains about 40 μg/ml of factor XII (104). The protein structure includes a binding site that attaches to negatively charged surfaces (105), and in vivo this surface is usually the phospholipid bilayer of an endothelial cell. In vitro, factor XII will bind to glass or other artificial surfaces with an anionic charge (103), which has given it the common name of 'glass activation factor'.

Another protein that is required for contact activation is plasma prekallikrein. Normal human plasma contains about 40 μg/ml of plasma prekallikrein, which circulates in an inactive form and is another serine protease in its active state. Plasma kallikrein then converts factor XII into factor XIIa that in turn activates more plasma kallikrein and amplifies factor XII activation. This is a form of positive feedback

within the coagulation process that increases the rate of contact activation via amplification of factor XIIa formation.

A third protein required for contact activation is factor XI. The inactive form of factor XI is composed of two homodimers, each containing a serine protease domain. Factor XIIa activates each monomer of the factor XI protein, which results in a 2:1 formation of catalytically active domains per factor XIa/XIIa molecule (106). This process, combined with the amplification step achieved through XIIa-mediated activation of plasma kallikrein, rapidly amplifies the rate of contact ativation.

The fourth protein that is required for contact activation is high molecular weight kininogen (HMWK). HMWK is a cofactor that binds plasma prekallikrein, factor XI, and bradykinin. Bradykinin is bound to plasma kallikrein, and the binding of HMWK to the bradykinin/plasma kallikrein complex promotes the conformational change of the bradykinin structure to form a heavy and light chain (107;108). Normal human plasma contains about 70-90 μg/ml (109;110), with the majority of plasma prekallikrein and factor XI bound to the light chain (111).

The final element required for contact activation is a negatively charged surface. As stated above, the phospholipid layer of a damaged endothelial cell can serve as the surface on which factor XII and the light chain of HMW kininogen bind. Because these proteins are circulating in plasma, it is necessary for them to bind in close proximity to one another (103). This allows factor XII and HMW kininogen (with plasma prekallikrein and factor XI bound to its light chain) to efficiently initiate the coagulation process in a localized region. In light of the many advances in the

understanding of the coagulation processes, it is still not fully understood how the process of contact activation is initiated in vivo.

Investigators have hypothesized that the binding of factor XII to the negative surface produces a conformational change in the structure of the protein that results in its activation (112). Another theory is that trace amounts of factor XIIa activate the process, either by binding to the cell membrane and converting enough plasma prekallikrein into plasma kallikrein, or binding in close enough proximity to the HMW kininogen complex to efficiently activate the coagulation process (113).

When factor XII is activated, it is able to exert its proteolytic properties on plasma prekallikrein to produce plasma kallikrein. This amplifies the amount of factor XIIa that is generated, which then converts factor XI into factor XIa, which is the end product of contact activation (103). Factor XIa then begins the intermediate phase of the intrinsic pathway with the activation of factor IX. Normal human plasma contains between 3-5 μg/ml of factor IX, which in its active form, is a serine protease (114;115). Factor IX is vitamin K dependent and requires the presence of calcium ions (Ca^{2+}) as a cofactor for activation. When the membrane-bound factor XIa that is complexed with HMW kininogen combines with Ca^{2+} and factor IX in the presence of vitamin K, it is able to convert factor IX into factor IXa (116).

The next step in the intrinsic pathway is the activation of factor X. It is a two-chained glycoprotein, which in its active form is a serine protease (103). Activation of this protein requires the "Xase" complex that is formed by the interaction of factor IXa and the cofactors of factor VIIIa, Ca^{2+}, and a negatively charged phospholipid surface (103). Ca^{2+} circulates freely in human plasma and the negatively charged

phospholipid surface is likely to be the same as that on which contact activation was initiated.

Factors IX and X can also be activated through the extrinsic pathway. A variety of cells in the human body, including endothelial cells, express surface tissue factor (TF). The natural arrangement of the phospholipid membrane of cells that express TF is asymmetrical, and following disturbance, the membrane protein, scramblase, intiates the rearrangement of phosphatidylcholine (PC) from the inner to the outer membrane. Undisturbed endothelial cells do not express PC on their outer membrane, however, the scramblase-mediated re-arrangement of PC allows the encrypted (inactive) form of TF to be activated.

TF is a glycoprotein receptor that binds circulating factor VII, and this process can occur with either active or inactive TF. Furthermore, the action of PC re-arrangement can activate TF/factor VII complex, or unbound TF. The de-encryption/activation of TF causes a conformational change in factor VII that exposes the catalytically active domain of factor VII, thus forming active factor VII. The resulting TF/factor VIIa complex then activates circulation factors IX and X, which then bind to the TF/factor VII complex, creating the catalytically active Xase complex. At this point, the intrinsic and extrinsic pathways converge. The Xase complex leads to thrombin activation and subsequent fibrin formation.

Between the steps of Xase and fibrin activation, there are several regulatory and amplification steps that occur. Factor VIII, which circulates in plasma in an inactive complex with a carrier protein called von Willebrand factor, is a cofactor in the amplification of Xase-mediated thrombin activation. Free plasma factor VIII is

vulnerable to premature degradation in plasma and it appears that von Willebrand factor serves to prevent its degradation. When the factor VIII/von Willebrand factor complex comes into contact with the Xase complex, factor VIII undergoes a conformational change that promotes its activation by thrombin. Following factor VIII activation, the von Willebrand carrier protein is capable of promoting coagulation by activating platelets and promoting platelet aggregation. Thus, activation of factor VIII can be viewed as a potent amplification step in the coagulation mechanism. Factor VIII activates thrombin, which in turn activates fibrin, factor V, and factor VIII and promotes platelet aggregation.

Fibrin circulates in its inactive form as fibrinogen and normal human plasma contains between 2 and 4.5 mg/ml of the zymogen, which greatly exceeds the coagulation proteins previously mentioned (103). Fibrin monomers serve as the structural component of a blood clot by cross-linking and forming the framework of fibers in which platelets bind. Stabilization of fibrin cross-linking requires factor XIII and Ca^{2+} as cofactors. Factor XIIIa is activated by thrombin, binds to the fibrin monomers in a calcium dependent manner, and stabilizes the cross-linked fibrin polymers resulting in a fibrin clot.

The formation of a blood clot is necessary to prevent infection and the loss of blood following vascular injury. When this occurs, fibrin is formed and stabilized through the mechanisms discussed above and it is the role of the fibrinolytic system to degrade, or lyse, the fibrin clot. The main protein involved in fibrin degradation is plasmin. It circulates in plasma in the inactive form called plasminogen, which binds

to the Aα chain of fibrin. Since the clot is bound to the membrane surface, the process of fibrinolysis is restricted to the site of fibrin formation.

The Aα chain of fibrin also contains a binding site for tissue plasminogen activator (tPA), which is the main plasma activator of plasminogen. When single-chained tPA binds to fibrin, it undergoes a conformational change that increases its proteolytic activity toward plasminogen. This serves to activate plasmin, which in turn degrades the fibrin proteins and converts the single-chained tPA to a more highly active double-chained tPA. The increased activity of double-chained tPA results in a greater amount of plasmin activation and increases the rate of fibrin degradation. The end product is the release of fibrin dimer proteins (FDP).

tPA is produced, stored and released from vascular endothelial cells. Normal human plasma contains about 3 μg/ml of tPA at rest (117). Studies have reported plasma levels to increase five-fold during exercise (118;119); the most probable mechanism for this elevation is an increase in vascular blood flow (120) and/or an increase in catecholamines (119). In the absence of injury, the increase in tPA release is matched by an increase in plasminogen activator inhibitor-1 (PAI-1) release.

Endothelial cells and blood platelets release PAI-1, which can circulate bound to fibrin or in a complex with vitronectin. Normal human plasma contains about 25-30 ng/ml of PAI-1, however it has been shown to increase with age (117;121). PAI-1 inhibits tPA by binding to it and blocking its proteolytic activity. This tPA-PAI-1 complex formation occurs quickly in plasma, which can result in a rapidly reduced fibrinolytic capacity.

As stated above, normal human plasma contains about of 3 μg/ml tPA, and about 13.5 AU/ml of PAI-1 at rest (122). As age increases, and/or with the onset of certain diseases like hypertension, diabetes, and hyperlipidemia, or nicotine usage, the PAI-1 concentration has been shown to be elevated at rest, and exhibit a less pronounced reduction during exercise compared to healthy controls (123). As with PAI-1 in the subject population above, tPA has been shown to be either decreased (123), or unchanged (121;124) at rest, with a concomitant decreased response during and after physical exertion compared to healthy controls.

In healthy individuals, a balance between thrombotic and fibrinolytic potentials maintains hemostasis. However, changes in coagulative and fibrinolytic potentials during and after exercise may cause an imbalance that favors thrombosis. tPA activity increases and PAI-1 activity decreases during exercise and returns to baseline within one hour (125). Factor VIII increases with exercise (126), and has been shown to remain elevated for more than one hour after exercise (44). An increased potential for coagulation without a concomitant elevation in the fibrinolytic potential increases the possibility of clot formation, and in CVD patients this may lead to stenosis, angina, myocardial infarction, or stroke.

Some of the hemostatic markers, or variables, that are typically measured in response to exercise are changes in the plasma concentrations of factor VIII, thrombin-antithrombin III complex (TAT), tissue plasminogen activator (tPA), and plasminogen activator inhibitor-1 (PAI-1). Several studies have reported elevated FVIII levels immediately after exercise (126-128) and one hour after exercise (125).

These increases have been observed in both high intensity, short duration, and lower intensity, long duration exercises.

The exact mechanisms responsible for the reported increases in FVIII following acute exercise are not completely understood, but appear to be related to catecholamines (129) and/or vascular endothelial cell damage (130). Cohen et. al. (129) reported a decreased FVIII response after propranolol infusion when compared to a placebo trial during a maximal treadmill exercise test. These investigators also found no significant difference in the fibrinolytic response in the placebo and propranolol infusion trials. This suggests that the stimulus for FVIII activation may be mediated by the β-adrenergic system, while the stimulus for fibrinolytic system activation may be dependent on other factors.

Factor VIII assists in the conversion of prothrombin into thrombin, which results in an enhanced state of hypercoaguability. Antithrombin III (AT III) is a protein that regulates thrombin formation, thus regulating coagulation. It circulates in plasma and binds to thrombin, creating the inactive thrombin-antithrombin III (TAT) complex. AT III is also effective in inhibiting the proteolytic activity of factors IXa, Xa, and XIIa. The formation of the TAT complex is a slow process that is enhanced when heparin, that is present on the surface of endothelial cells, binds to AT III, which increases its affinity for thrombin. Fibrin formation from fibrinogen via thrombin remains possible in the presence of AT III because the speed at which thrombin exerts it enzymatic cleavage of fibrinogen exceeds that of the TAT complex formation.

Several studies have reported elevations in the TAT complex following exercise (120;130;131). Bartsch et. al. (131) reported in an investigation of Swiss long distance runners, that an increase in thrombin generation occurred in vivo, following a 100 km race, as reflected by increased TAT levels following the race. Other studies have shown that increases in TAT, which indicates thrombin generation, can be observed in exercise of a shorter duration and coincides with increases in prothrombin fragments 1+2 (PTF 1+2) (130;132).

Thrombin can act as a potent stimulator of coagulation by activating proteins within the coagulation pathway, enhancing platelet stimulation and aggregation, and converting fibrinogen into fibrin. However, in the presence of thrombomodulin, thrombin can become an effective anti-coagulant. Thrombomodulin is a high affinity protein receptor for thrombin on the surface membrane of endothelial cells. When thrombin binds to thrombomodulin, it results in a conformational change in the thrombin molecule (103). In the presence of Vitamin K, this conformational change allows activation of protein C (133).

In healthy individuals, exercise results in an increased potential for coagulation that rarely leads to fibrin formation (43;131). The increased potential for coagulation is the result of an increased activity of the intermediate enzymes within the pathway and an increase in platelet adhesiveness (134). The increased activity is well regulated and prevented from producing excessive or inappropriate thrombi through the activity of the regulatory proteins within the coagulation pathway. This allows activation of the intermediate coagulation enzymes without the activation of fibrin.

Atherosclerosis is now considered to be a low-grade inflammatory disease that results in endothelial cell dysfunction. Recent evidence has shown that several factors, including hyperinsulinemia and hypertriglyceridemia, whether chronic or postprandial, is associated with leukocyte activation and an elevated potential for thrombosis which promotes endothelial cell dysfunction and vascular damage. Although the underlying mechanisms are not completely understood, it has been shown in monocyte and endothelial cell cultures incubated with triglyceride (TG) rich chylomicrons and VLDL, that FFA and TG uptake activates FVII, TF and PAI-1.

It is logical to assume that not all sections of the vascular endothelium respond to an oral fat load in the same manner. In an older, sedentary population, there is likely to be moderate levels of atherosclerosis present in coronary and/or cerebral arteries, where the risk of thrombus formation is much higher than in other, non-atherosclerotic arteries. The underlying, low-level inflammatory state in diseased arteries is capable of attracting monocytes, which can be a potent stimulus for increased coagulation and decreased fibrinolysis. Furthermore, the majority of life is spent in the postprandial state, and for these reasons, the necessity of an investigation into the local fibrinolytic response in conjunction with the systemic response is apparent.

Recent studies have shown that a positive feedback mechanism within the hemostatic system is able to paradoxically contribute to the progression of plaque formation via platelet activation. The thrombogenic surface of the fibrous cap and the intimal layers of atherosclerotic regions within the arteries are capable of activating platelets. Activated platelets release pro-atherogenic factors, which creates a

deleterious positive feedback mechanism that destabilizes the fibrous cap. Therefore, mechanisms for reducing or inhibiting platelet activation would be highly effective in reducing the progression of atherosclerosis.

Atherosclerosis is a long-term, complex disease that involves many factors and has been shown to begin very early in life (135). Although complications of the disease typically do not manifest until the fourth or fifth decade of life, foam cell formation and fatty streaks have been shown in autopsies of healthy adolescents (136-138). Foam cells are lipid-laden macrophages that have migrated into the subendothelial matrix of the vascular bed in order to scavenge lipids and lipoproteins that have undergone oxidative modification by the endothelium (139). The accumulation of foam cells and the resulting inflammatory response within the intimal layer of the subendothelial space eventually creates a fatty streak, which is characterized by endothelial cell dysfunction, smooth muscle cell migration and proliferation, and collagen formation (140;141). Atherosclerotic lesions begin to appear when the underlying area of the fatty streak becomes raised as foam cells and smooth muscle cells are deposited, which begins to partially occlude the vessel. Finally, the fibrous cap of the atherosclerotic lesion weakens due to the apoptotic regression of smooth muscle cells via tumor necrosis factor-α (TNF-α) and chymase which are released from macrophages (142).

Total plasma high density lipoprotein (HDL) has been associated with a reduction in the relative risk for CVD, and it can be reduced into two main sub-classes: HDL_2 and HDL_3. The former is the lipid-rich form that transports scavenged lipids and lipoproteins from the circulation to various tissues within the body

(143;144). The lipids and lipoproteins carried by HDL_2 are transferred to other cells through various surface receptor enzymes such as lipoprotein lipase (LPL), hepatic lipase (HL), and endothelial lipase (EL). Following the removal of lipids, HDL_2 becomes denser due to a higher relative apolipoprotein content and lower lipid content, and is thus classified as HDL_3 (143). The lipid-free HDL_3 is able to further scavenge deposited lipids and cholesterol, and as the relative lipid content increases, the HDL_3 again becomes HDL_2. This cyclic process, termed reverse cholesterol transport (RCT), is the most widely accepted mechanism for HDL mediated CVD protection (145-147). However, it also appears that the lipid-free form of HDL is the sub-fraction that has the greatest platelet inhibitory effect as well. HDL_3 has been shown to directly supply arachadonate to endothelial cells in vitro (147). It is unknown if this functions *in vivo*, although HDL has been correlated with plasma levels of stable PGI_2 metabolites (147) and in conjunction with its effects in RCT, providing the substrate for PGI_2 synthesis is another proposed mechanism for HDL-mediated CVD protection.

Diets high in fat have also been associated with the development of CVD, and RCT is activated following ingestion of a high fat meal (145). Intestinally absorbed free fatty acids are carried within chylomicrons and very-low density lipoproteins (VLDL) as triglycerides (TG) and delivered to tissues throughout the body. After the removal of TG, the remnant TG and lipoproteins of chylomicrons and VLDL are scavenged by HDL_3 through reverse cholesterol transport. It would be expected that following a high fat meal, plasma HDL_3 concentrations would be lower due to their increased conversion into HDL_2, which has been confirmed by Lassel and

coworkers (148). The reduction in HDL_3 is most likely due to the increased activity of LPL, HL, and/or EL during RCT. These three enzymes are the major RCT mechanisms for TG and HDL, although compared to LPL and HL, EL has lower TG activity and higher Apo A-I phospholipid activity.

Platelets are vital to the progression of atherosclerosis because they are responsible for mediating the smooth muscle cell migration into the intimal layer. Platelets are anucleated blood cells derived from megakaryocytes that participate in hemostasis and wound repair by binding to integrins expressed on damaged or dysfunctional endothelial cells which mediate their activation. Platelet derived growth factor (PDGF) is released from activated platelets in order to facilitate tissue repair and wound healing. Platelets also provide a negatively charged phospholipid surface for Factor X and prothrombin activation reactions. Furthermore, they release substances such as serotonin that mediate blood vessel contraction and synthesize and release thromboxane A2 (TxA_2). Thrombin and TxA_2 are the most potent platelet agonists acting at the site of injury on their respective platelet receptors (140;141;149-151); thrombin activates platelets and TxA_2 causes platelets to aggregate, thus providing a positive feedback mechanism for continued platelet activation. The aggregated platelets continue to synthesize and release these factors until platelet retracting factors such as prostaglandin I_2 (PGI_2) are synthesized (149).

PGI_2, or prostacyclin, is a metabolite of arachadonic acid which is formed in platelets and endothelial cells as well as several other cell types. Arachidonate is derived from phospholipids of cellular membranes or from exogenous sources such as HDL (147). The conversion of arachadonate to PGI_2 is mediated by the rate limiting

enzyme cyclooxygenase (COX) (146;147;150). COX is expressed in two different isoforms, COX1 and COX2, the former being expressed in most tissues, and the latter is typically absent, but its expression is induced following stimulation by cytokines and other mediators (146). Animal studies have shown that increases in aortic smooth muscle cell release of PGI_2 is dependent on HDL mediated increases in COX2 transcription and translation (149;150). PGI_2 is the most potent inhibitor of platelet aggregation known and it also serves to disperse existing aggregates (151). It acts by increasing adenylate cyclase and other second messengers within the platelet, although it has relatively short half-life of 30 minutes (151). Due to the inherent instability of PGI_2 in plasma, the capacity for prolonging its effect is as important in the development of atherosclerosis as its formation.

HDL_3 has been shown to enhance PGI_2 activation and stabilization by endothelial cells (152), and Apo A-I serves as the PGI_2 stabilizing factor. Chronic aerobic exercise training has been shown to increase Apo A-I levels (153), which are generally carried in plasma in the form of total HDL or its sub-fractions. Therefore, having a larger amount of Apo A-I due to exercise training would provide for a larger pool of arachadonate from increased plasma HDL levels, as well as a prolonged half-life of PGI_2 due to an increased concentration of PGI_2 stabilizing factor.

Summary

Chronic hypertryglyceridemia is thought to be atherogenic and is associated with an elevated thrombotic potential (154-156). Research has shown that individuals with elevated TG have higher levels of FVIIa (157-159), TF (160) and PAI-1 activity/antigen (161;162), with lower tissue plasminogen activator (tPA) activity

than healthy controls (162). The mechanisms responsible for the activation of coagulation and the reduced fibrinolytic capacity are believed to be due to the negative charge of fatty acids (7;163) and the constituent lipoproteins of low density lipoproteins, VLDL, and chylomicrons (164;165). Additionally, it has been shown that VLDL binding to its endothelial cell receptor results in increased transcription and translation of the PAI-1 gene (7).

It would be expected that an acute oral fat load would increase the thrombotic potential due to a higher fatty acid content of LDL, VLDL, and chylomicrons circulating in the blood. This has been confirmed by postprandial lipemia studies in which FVII levels were elevated for up to 8 hours following the ingestion of a high fat meal (166-168). Furthermore, the standard liquid fat meal (30) is composed of a large amount of fat, with moderate amounts of sugar and protein, which have been shown to cause a concomitant increase in insulin. When insulin binds to its receptor, it causes the release of stored PAI-1 in cultured human umbilical vein endothelial cells (169) and activates transcription and translation to increase PAI-1 synthesis (161). Therefore, it is apparent that the combined effect of elevated TG and insulin following postprandial lipemia should increase PAI-1 levels following a high fat meal. However, in vivo studies have shown a paradoxical decrease in the circulating levels of PAI-1 following a high-fat meal (162;167), and it is the purpose of this study to determine if local PAI-1 production, as evidenced through leukocyte gene transcription, is increased during postprandial lipemia, despite the fact that systemic levels are decreased.

The postprandial decrease in systemic PAI-1 activity suggests that there is an acute cardio-protective effect following ingestion of an oral fat load, however it is extremely unlikely that this would occur within the areas of the endothelium that are atherosclerotic. It is probable that within the local areas of the endothelium that are predisposed to atherosclerosis, coagulation and PAI-1 activity would be higher than the total systemic activity, which would negate the possibility of a cardio-protective effect from lower systemic PAI-1 activity. The most likely explanation for the decrease in PAI-1 activity is the increase in hepatic blood flow after the meal. PAI-1 is cleared from plasma through an interaction with the hepatic LDL receptor, and in response to an alimentary meal, a concomitant increase in PAI-1 clearance rate would occur when the rate of hepatic blood flow increases. Kemme and colleagues (170) found that when FFA was injected intravenously, there was no change in tPA or PAI-1 concentration because the postprandial increase in hepatic blood flow was absent. Although this illustrates the point that systemic fibrinolysis is related to hepatic blood flow, it fails to show how local fibrinolysis (i.e. at the endothelial cell surface) is affected by FFA uptake, because the intravenous injection of TG and/or FFA circumvents the natural intestinal absorption of lipids and prevents their incorporation into chylomicrons and VLDL (73;171). Therefore, when measuring fibrinolysis, the complex nature of the postprandial state requires a more thorough evaluation of the in vivo fibrinolytic response than has been conducted to date.

When looking at systemic fibrinolysis, it is difficult to determine the separate effects of the increased lipid content of cholesterol versus increased hepatic blood flow. However, if a measure of local fibrinolytic response is incorporated, a more

thorough description of the postprandial events may be achieved. This can be accomplished by measuring IL-6, TNF-α, and PAI-1 gene expression in leukocytes because they are recruited by activated endothelial cells. VLDL has been shown to bind to and activate endothelial cells, which causes an increase in the expression of vascular and intracellular adhesion molecules (VCAM) (ICAM-1). These proteins bind circulating leukocytes and arrest them on the endothelial cell surface where they become activated.

Chylomicrons enter the circulation through the lymphatic system (73;171) where they are capable of interacting with a large number of leukocytes, and TG-rich lipoproteins binding to leukocytes have been shown to increase PAI-1 and TF gene expression in vitro. In light of these results, it is possible that local fibrinolysis, as evidenced by PAI-1 gene expression in leukocytes, could be decreased even though there is a concomitant increase in systemic fibrinolysis. This is because prior to the VCAM/ICAM-mediated binding of leukocytes to the endothelial cell surface, circulating leukocytes become activated, and it is at this point where we believe that changes in PAI-1 gene transcription will be evident. A major limitation to the current study design is our attempt to measure activated leukocytes within the plasma circulation, and while it is logical to assume that a large portion of activated leukocytes are tethered to the endothelium, there is strong evidence to suggest that activation products (i.e. IL-6 and TNF-α) contribute to further leukocyte activation and coagulation (172;173). Furthermore, while we are limited in our ability to determine the contributions and extent to which endothelial bound leukocytes affect hemostasis, we can determine the contribution of circulating leukocytes on the

fibrinolytic response by measuring PAI-1 gene expression. In order to verify that activated leukocytes are present in our samples, we intend to measure the changes in IL-6 and TNF-α gene expression in conjunction with PAI-1, with the belief that any change in activation observed in our samples would most likely be higher within the endothelial bound pool.

We believe that the extent to which leukocyte activation occurs following aerobic exercise training will be lower than that which can be seen prior to training. It has been shown that following an acute bout of exercise, plasma IL-6 levels were significantly lower 4-hours after ingestion of a high fat meal, compared to a non-exercise control (174). Additionally, while Gill et. al. (175) reported that after a period following the cessation of exercise training, there were no significant postprandial changes in TNF-α levels, but the average TNF-α levels were 35% higher after the cessation of exercise training compared to the trained state. Based on these results, it is logical to assume that ingestion of a high fat meal will increase leukocyte activation, and that aerobic exercise training will reduce the extent to which leukocyte activation occurs.

Based on the information outlined above, it is logical to assume that not all sections of the vascular endothelium respond to an oral fat load in the same manner. In an older, sedentary population, there is likely to be some degree of atherosclerosis present in coronary and/or cerebral arteries (176), where the risk of thrombus formation is much higher than in other, non-atherosclerotic arteries (177). The underlying, low-level inflammatory state in diseased arteries is capable of activating leukocytes, which can be a potent stimulus for increased coagulation and decreased

fibrinolysis. There is no information on the effect of exercise training on coagulation potential of fibrinolytic activity during the postprandial state, as all studies have tested subjects while fasting. However, the majority of life is spent in the postprandial state, and for this reason, the necessity of an investigation into the local fibrinolytic response, in conjunction with the systemic response, is apparent.

Aerobic exercise training has been shown to reduce the overall risk for CVD-related outcomes through several distinct mechanisms, one of which is an improvement in lipoprotein lipase (LPL) activity. Physical activity has been associated with an increased clearance of FFA and TG following ingestion of an oral fat load (13;14), and in a cross-sectional study Merril and colleagues (178) found that peak TG and total lipemic response were lower in young, endurance trained men compared to sedentary controls independent of fasting TG levels. A well documented effect of aerobic exercise training is an increase in muscle capillary density, which is believed to be responsible for a large portion of the increase in LPL activity. In an 8-week single leg training model, capillary density increased in the trained leg by 20%, and LPL activity was 70% higher than in the non-trained control leg (179). One result of increased LPL activity following exercise training is an increase in the clearance rate of circulating TG which provides a mechanism for an increased tolerance to fat consumed in the diet.

Not all studies have shown an increase in the rate of TG clearance with exercise training. This is probably due to the fact that the intensity of exercise and/or quantity of energy expenditure during exercise is responsible for the magnitude of change in TG clearance. This factor is best illustrated by comparing the results of

postprandial lipemia tests following acute exercise versus a long-term training study. In one acute exercise study, subjects exercised for 2-hours at 31% VO_2max and experienced a 31% reduction in lipemic response the following day (29). In a 12-week training study, women engaged in unsupervised brisk walking for 21 min/day and the lipemic response was measured before and after training, and there was no difference in peak TG concentration or in the total lipemic response (38). The difference between these two studies is likely do to the lower caloric expenditure in the latter study. In our laboratory however, we have found that in subjects the same as those to be enrolled in this proposed trial, a 6-month supervised aerobic exercise training program at 70% VO_2max reduced TG area under the curve (AUC) by 40% and FFA AUC by 33% following a high-fat meal (unpublished data). These results illustrate the need for a well-structured and controlled intervention to optimize the training effect on increased FFA and TG clearance.

As stated above, the potential for coagulation increases during postprandial lipemia, which is believed to be due to the increased TG content of chylomicrons and VLDL. FVII and TF were shown to increase in response to a high fat meal, due to an activation of the intrinsic coagulation pathway without an increase in thrombin-antithrombin III (TAT) levels (15). The increase in the potential for coagulation rarely leads to thrombus formation, which suggests that coagulation is being inhibited, probably by tissue factor pathway inhibitor (TFPI). Thus, if a change in FVIIa is to be measured during a postprandial lipemia test, then the change in TFPI activity should be included as well in order to provide a complete picture of the response of the coagulation profile.

If exercise training reduces the peak and total lipemic response after a high fat meal, then it is logical to assume that it would decrease the potential for coagulation and leukocyte PAI-1 gene expression. An evaluation of these hypotheses would help explain the disparity in the results of exercise training on hemostasis, where currently there is no consensus as to whether exercise training improves hemostatic profiles. Many studies have reported lower fibrinogen fragments or thrombin levels in physically active individuals, but the results of longitudinal exercise training studies have not been as clear. Some have reported small increases in tPA antigen, or lower FVIIa, and thrombin activation, while others have shown no change at all. Furthermore, the magnitude of change with exercise training is likely to be dependent on the volume and/or intensity of exercise.

In studies conducted previously in our laboratory, we have found that the exercise training-induced changes in PAI-1 activity (15.6 ±1.4 to 12.9 ±1.4 AU/ml) ($p=0.03$) and tPA activity (0.54 ±0.10 to 0.92 ±0.10 IU/ml) ($p=0.007$) in 58.3 ±1.3 year old men were significant. Additionally, we have found that FVIIa decreased from 106.7 ±1.4 to 104.2 ±1.4 % normal ($p=0.005$) in men and women at rest. Thus, we have demonstrated an 18% decrease in PAI-1 activity, a 70% increase in tPA activity (unpublished data), and a 2.5% decrease in FVII:ag (180) using a supervised, 6-month exercise training intervention at 70% VO_2max, which provides further support for the necessity of a well-structured, prolonged, and controlled intervention, such as the program that we currently employ, to optimize the training effect on inflammation, coagulation, and fibrinolysis.

While we have demonstrated an improvement in coagulation and fibrinolysis at rest, few cardiovascular and cerebrovascular thrombotic events occur in the fasted or resting state. Because of this, there is a critical need to assess hemostasis following a challenge such as the postprandial lipemia test. Such a test provides an accurate and reproducible method of challenging a system that is designed to respond to a stimulus, and when incorporating exercise training, the ability to determine if training improves the hemostatic profile would be enhanced. Furthermore, this in vivo model could partially explain the discrepancy between the in vitro and in vivo results of previous studies on changes in PAI-1 concentrations following a high fat meal. Since vascular thrombosis is responsible for the majority of CVD- and stroke-related events, the investigation of these hypotheses will lead to a better understanding of the methods by which exercise training reduces CVD- and stroke-related morbidity and mortality.

Appendix H: List of Abbreviations

1) **AGE-** Advanced glycosylation end products
2) **AT III-** Antithrombin III
3) **AUC-** Area under the curve
4) **BSA-** Body surface area
5) **CAT-** Carnitine acyl transferase
6) **CCK-** Cholecystokinin
7) **CHO-** Carbohydrate
8) **CM-** Chylomicron
9) **CoA-SH-** Coenzyme A
10) **COX-** Cyclooxygenase
11) **CT-** Computed tomography
12) **CVD-** Cardiovascular disease
13) **DAG-** Diacylglycerol
14) **EL-** Endothelial lipase
15) **FABP-** Fatty acid binding protein
16) **FABP(PM)-** Plasma membrane fatty acid binding protein
17) **FAT/CD-36-** Fatty acid translocase
18) **FATP-1-** Fatty acid transport protein-1
19) **FDP-** Fibrin dimmer proteins
20) **FFA-** Free fatty acid
21) **FVIIa-** Activated factor VII

22) **Glu-** Glucose

23) **HCO_3^- -** Bicarbonate

24) **HDL-** High density lipoprotein

25) **HMWK-** High molecular weight kininogen

26) **HRT-** Hormone replacement therapy

27) **hsCRP-** High sensitivity C-reactive protein

28) **HSL-** Hormone sensitive lipase

29) **HSPG-** Heparin sulfate proteoglycans

30) **ICAM-1-** Intracellular adhesion molecule-1

31) **IL-6-** Interleukin-6

32) **Ins-** Insulin

33) **IRS-1-** Insulin receptor substrate-1

34) **KO-** Knock out

35) **LDL-** Low density lipoprotein

36) **LDLR-** Low density lipoprotein receptor

37) **LP-**lamina propria

38) **LPL-** Lipoprotein lipase

39) **LRP-** Low density lipoprotein related receptor

40) **MAG-** Monoacylglycerol

41) **MTP-** Microsomal triglyceride transfer protein

42) **NAC-** No amplification control

43) **NTC-** No template control

44) **PAI-1-** Plasminogen activator inhibitor-1

45) **PC-** Phosphatidyl choline

46) **PDGF-** Platelet derived growth factor

47) **PDK1**- Phosphatidylinositol-dependent kinase-1

48) **PGI_2-** Prostaglandin I_2

49) **PI3K-** Phosphatidyl $inositol_3$ kinase

50) **PIP_3-** Phosphatidylinositol-3,4,5-triphosphate

51) **PKA-** Protein kinase A

52) **PL-** Phospholipid

53) **PPAR-** Peroxisome proliferator activated receptor

54) **PPL-**Post prandial lipemia

55) **PPLT-** Post prandial lipemia test

56) **PTF 1+2-** Prothrombin fragments 1 and 2

57) **RAP-** α_2-Macroglobulin receptor associated protein

58) **RCMP-** Remnant chylomicron particle

59) **RCT-** Reverse cholesterol transport

60) **RER-** Rough endoplasmic reticulum

61) **RNA pol II-** Ribonucleic acid polymerase II

62) **rt-PCR-** Reverse transcription polymerase chain reaction

63) **SER-** Smooth endoplasmic reticulum

64) **TAT-** Thrombin-Antithrombin III complex

65) **TEE-** Total energy expenditure

66) **TF-** Tissue Factor

67) **TFPI-** Tissue factor pathway inhibitor

68) **TG-**Triglyceride

69) **TNF-α-** Tumor necrosis factor alpha

70) **tPA-** Tissue plasminogen activator

71) **TxA_2-** Thromboxane A_2

72) **VCAM-1-** Vascular cell adhesion molecule-1

73) **VLDL-** Very low density lipoprotein

74) **Xa-** Activated factor X

Appendix I: References

Reference List

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